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ORIGINAL ARTICLES

THE EFFECT OF BRONCHIAL ASTHMA ON THE HEART, WITH SPECIAL REFERENCE TO THE SPATIAL VECTORCARDIOGRAM

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DOES long-standing bronchial asthma have an effect on the heart? Various investigators have arrived at diametrically opposite conclusions in answering this question. The present study, which has special reference to spatial vectorcardiography in bronchial asthma, is undertaken because this technique, a comparatively new tool in cardiac diagnosis, offers information which reflects alterations in the myocardium.

HISTORICAL REVIEW

In 1927 Kahn,[1] in studying fifty cases of bronchial asthma, observed ten instances of right ventricular preponderance, twenty-one cases of left ventricular preponderance, and nineteen normal electrocardiograms. There was no evidence of myocardial involvement. Alexander and associates[2] found, in the fifty patients they studied, that bronchial asthma produced no changes indicative of myocardial damage. Unger,[3] in 1932, concluded from an electrocardiographic study of seventy-four cases of bronchial asthma that there was evidence of "myocardial damage" in his patients. It should be noted, however, that in this report the only basis for such a dignosis is the presence of mild conduction disturbances and of right ventricular preponderance. However, one of us[4] found in the same year no clinical or electrocardiographic evidence of permanent cardiac damage in fifty cases of long-standing bronchial asthma. Minor transitory ST interval and T wave alterations occurred during the acute, severe paroxysms in some of these patients. It was thought that these resulted from the associated transient asphyxia. Similar transient electrocardiographic abnormalities are reported in serum sickness and other types of acute allergic

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reactions.[5-12] Because of the latent period, the short duration, and the apparent reversal of these changes by antihistaminics, it is thought by some[13] that these changes are not entirely due to anoxia.

Colton and Ziskin[14] subsequently reported eleven abnormal electrocardiograms in fifty-six cases of bronchial asthma. Harkavy and Romanoff[15] found definite electrocardiographic changes in bronchial asthma. Urbach[16] found electrocardiographic evidence of "myocardial involvement" in sixty-two, or 29 per cent, of 209 cases of bronchial asthma during the asthma-free period. It is to be pointed out, however, that in many of these references the only basis for the diagnosis of an electrocardiographic abnormality was the presence of minor changes, such as left axis deviation. Schiller and colleagues[17] demonstrated electrocardiographic abnormalities in cor pulmonale associated with bronchial asthma. Rom and Jend,[18] investigating fifty cases of bronchial asthma using the twelve-lead electrocardiogram, found that 88 per cent were normal and that 12 per cent presented such abnormalities as P pulmonale, right ventricular hypertrophy, and some T wave variation.

Transient alterations in the cardiogram, such as those encountered in acute clinical allergic episodes, may be seen also in the experimental animal. One of us[19] obtained ST and T wave changes indicative of myocardial ischemia during anaphylactic shock in the guinea pig. In the same paper it was indicated that these findings were identical with those which occur in guinea pigs as a result of experimental asphyxia[19] and in experimentally induced histamine shock.[20] Others produced similar electrocardiographic changes during anaphylaxis in the rabbit.[21]

MATERIAL AND METHOD OF STUDY

A total of 177 asthmatic patients are included in this study. These patients show either a present or a past clinical picture of an atopic background. In so far as it was possible, patients in whom a definite diagnosis of bronchial asthma could not be made were excluded. We realize the frequency and difficulty of making a differential diagnosis between chronic obstructive pulmonary emphysema and allergic bronchial asthma.[22] Each patient was subjected to a complete, adequate medical and allergic investigation. This included, in addition to routine clinical and laboratory tests, cardiac measurements obtained from roentgenograms of the heart and x-ray studies of the pulmonary fields. Roentgenograms were done in each case on both inspiration and expiration in the posteroanterior and lateral positions. By superimposing these plates, it is possible to gauge the degree of expansion and contraction of the pulmonary fields and to determine in an approximate way the degree of residual air present in the lung. In addition, fluoroscopic examinations ascertained the movements of the diaphragm. It was noticed that in the presence of emphysema there occurred a definite slowing in the diaphragmatic movements following forcible expiration; at times the diaphragm seemed almost immobile. Bronchiectasis was diagnosed on clinical grounds, on the basis of x-rays of the chest, and, when indicated, by bronchoscopic and bronchographic examinations. A twelve-lead electrocardiogram and a vectorcardiogram were performed on each patient.

The vectorcardiographic technique in our laboratory utilizes the cube system of electrode placement (Fig. 1) as devised by Grishman.[23, 24] The basis for this cube system involves the premise that the heart acts as a point source of electromotive forces within the center of a geometric figure, the cube. The electrodes defining this cube are placed on the thorax so that they are located approximately at points equidistant from the center. Fig. 1 represents diagrammatically the superimposition of the cube on the thorax. Numbers indicate

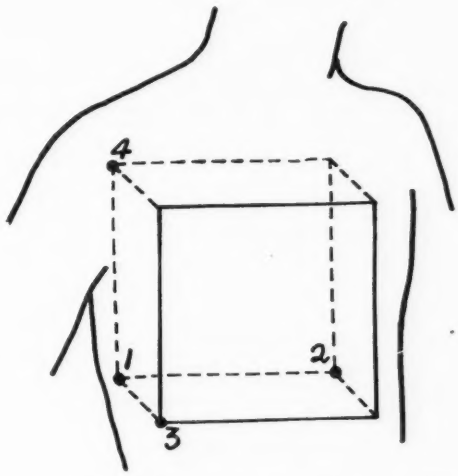

Fig. 1.—Superimposition upon the thorax of Grishman's cube. Numbers refer to electrode positions. (From Grishman, Borun, and Jaffe: Am. Heart J. 41: 483, 1951.)

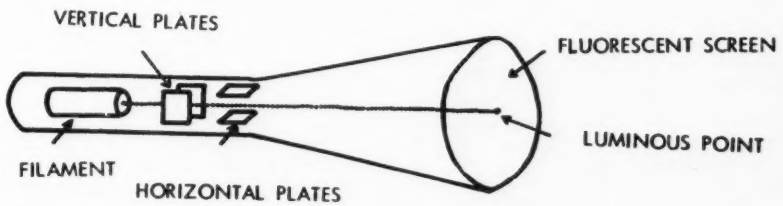

Fig. 2.—Diagram of cathode-ray tube containing a filament that emits electrons. Electron beam (dotted line) passes through an aperture in a positively charged grid and impinges on fluorescent screen as a luminous point. Vertical and horizontal plates deflect the beam as described in text. (From Grishman and Scherlis: Spatial Cardiography, Philadelphia, 1952, W. B. Saunders Company.)

electrode positions on the chest wall. These electrodes are so connected as to make it possible to record that portion of the single electrical field surrounding the heart that falls onto the three natural planes of the body. These planar projections as represented by vector loops, which will be described later, were viewed on the screen of the oscilloscope, appropriately scanned, and photographed (Fig. 2).

The summary of the clinical and laboratory data thus obtained was then placed on a properly coded table (Table I), from which it was transcribed to I.B.M. cards. The desired information, in relation to various combinations of items, was obtained by processing the punched cards through an I.B.M. machine.

TABLE I. SUMMARY OF CLINICAL, ELECTROCARDIOGRAPHIC, AND

	Clinical Data						
Case No.—							
Sex	(1) Male	(2) Female					1
Age	(1) 4-10	(2) 10-20	(3) 20-40	(4) 40-60	(5) 60+		2
Diagnosis	(0) No	(1) Asthma					3
	(0) No	(1) Chronic bronchitis					4
	(0) No	(1) Pulmonary emphysema					5
	(0) No	(1) Pulmonary fibrosis					6
	(0) No	(1) Bronchiectasis					7
Severity	(1) Mild	(2) Moderate	(3) Marked				8
Duration of disease	(1) 1-5	(2) 6-10	(3) 10+				9
Treatment	(0) None	(1) Symptomatic	(2) Allergy	(3) None	(4) Both		10
Duration of treatment	(1) 1-5	(2) 6-10	(3) 10+				11
Clinical cardiac diagnosis	(0) No	(1) Coronary					12
	(0) No	(1) Hypertension					13
	(0) No	(1) Rheumatic					14
	(0) No	(1) Cor pulmonale					15
	(1) No clinical evidence of cardiac disease						16
Clinical evidence of arrhythmia	(0) No	(1) S. arrhythmia					17
	(0) No	(1) P.B.					18
	(0) No	(1) A.F.					19
	(0) No	(1) Partial ht. blk.	(2) Complete				20
	(0) No	(1) Bradycardia					21
	(0) No	(1) Flutter					22
Failure	(0) None	(1) Cong. failure	(2) Angina	(3) Both			23
Enlarged	(0) None	(1) Enlargement-both	(2) Rt.	(3) Lt.			24
	(0) None	(1) Renal failure	(2) Electrolyte chgs.	(3) Both			25
Drugs	(0) No	(1) Digitalis					26
	(0) No	(1) Quinidine					27

VECTORCARDIOGRAPHIC DATA, PROPERLY CODED FOR TRANSCRIPTION TO I.B.M. CARDS

	Electrocardiographic Data	
Infarction	(0) No ant. infarct. (1) Ant. old (2) Ant. recent	28
	(0) None (1) Post. old (2) Post. recent	29
	(0) None (1) Diaphrag. old (2) Diaphrag. recent	30
Hypertrophy	(0) No (1) Rt. V.H. (2) Lt. V.H.	31
	(0) None (1) Pericarditis	32
	(0) None (1) Electrolyte changes	33
ST	(0) None (1) Nonspecific ST changes	34
	(0) None (1) C.I.	35
	(0) None (1) Embolism	36
Rhythm	(0) No (1) Sinus	37
	(0) None (1) P.B.	38
	(0) No (1) Sinus arrhythmia phasic (2) Sinus arrhy. nonphasic	39
	(0) None (1) A.F.	40
	(0) None (1) Bradycardia	41
	(0) No block (1) Part. L.B.B.B. (2) Comp. L.B.B.B. (3) Part. R.B.B.B. (4) Comp. R.B.B.B. (5) A.V.	42
P-wave	(0) No P-wave change (1) P. pulmonale (2) P. mitral	43
ECG diagnosis	(1) Normal (2) Abnormal (3) Probably normal (4) Prob. abn.	44
	Vectorcardiographic Data	
RSR¹	(1) Post. pip (2) R.B.B.B. (3) RVP (4) Post. infarct. (5) LVV (6) RVP + cond. delay	45
Increased R/SV1	(1) RVP (2) Posterior infarct. (3) Juvenile pattern	46
RSR¹ in V1	(0) None (1) Post. pip	47
Tall R in V1	(1) RVP (2) Juvenile pattern	48
	(0) No (1) Post infarction	49
	(0) No (1) RBB	50
	(0) No (1) LVP	51
	(0) None (1) Terminal cond. delay	52

(Cont'd on next page)

TABLE I. CONT'D

RVP types	1	2	3	4	5					53
Q2Q3	(0) Not significant				(1) Diaphragmatic infarct.					54
QaVF	(0) Not significant				(1) Diaphragmatic infarct.					55
	(1) WPW	(2) No								56
ST vector	(1) Pericarditis		(2) L.V.V.V.		(3) LVP	(4) CI	(5) Digitalis			
	(6) Rt. B.B.B.		(7) Nonspecific							57
T loop	(0) None	(1) Primary								58
	(0) None	(1) T-loop change								59
	(0) None			(1) Increased angular deviation						60
RS in all V leads	(1) Rotation	(2) RVP	(3) LVP							61
RS in all V leads	(0) None	(1) Infarction								62
P-loop	(1) RAP	(2) LAP								63
QS in V1	(1) Rotation		(2) A-S myoc. inf.		(3) LVP	(4) Isoelectr. R.				64
Sup. elec. field	(1) Normal ECG			(2) Abnormal ECG						65
	(1) VCG-RVP									66
	(0) None	(3) S wave	V5V6							67
	(0) None	(1) VCG-LVP								68
	(0) None		(1) Cond. delay term.							69
	(0) None		(1) Cond. delay mid.							70
	(0) None		(1) Cond. delay early							71
	(0) None	(1) L.B.B.B.								72
	(0) None	(1) LVP								73
Conclusion	(1) Critical import.		(2) Noncontrib.		(3) Clari. of ques. ECG			(4) Normal		74
	(1) Normal	(2) Abnormal		(3) Probably N.		(4) Probably abn.				75
Normal ECG (rs in V1)	(1) Posterior pip		(2) None							76
Myocardial infarction	(1) Antero-septal		(2) Anterior		(3) None					77
Axis deviation	(1) 0-60	(2) 60-90	(3) 90 to ±180		(4) 0– –90					78

THE VECTOR CONCEPT

It is impossible to present here an adequate and detailed explanation of the vector concept. This may be readily found in the literature.[23, 24] We shall be content, however, to offer only some of the basic principles, an understanding of which is essential to a proper evaluation of the results to be reported. The principle involved in vectorcardiography is similar to that which forms the basis for electrocardiography. The electrocardiogram represents the changes in electrical potential in the heart muscle which accompanies the spread of the excitation wave through the myocardium and which immediately precedes myocardial contraction. However, each electrocardiographic lead only mirrors that single portion of the total cardiac electrical field which is related to its own particular electrode position on the body surface. Each set of electrocardiographic leads is intimately associated with one of the three planar projections of the vectorcardiogram, projections which will be explained and described below. The six extremity leads, namely, the three standard leads and the three unipolar leads, record the electrical events occurring in the frontal plane of the body. The precordial, chest, or V leads are derived from the horizontal planar projection. Thus, the information provided by the electrocardiogram regarding the electrical activity of the heart is somewhat limited. The vectorcardiogram, because it represents the total electrical field surrounding the heart, thus offers more complete information.

The phenomena involving the electrophysiology of the heart may be summarized as follows: The syncytial myocardial unit in its resting, uninjured state has an equal number of ionic charges on either side of its covering membrane—positive on the outside and negative on the inside. In this resting state, the muscle unit has no electric field around it and is said to be polarized. As the muscle is stimulated, there occurs a change in surface membrane permeability and a consequent flow of negative electric charges to the outside (depolarization process). This results in the close apposition of positive and negative charges, in other words, the creation of dipoles. Each dipole is surrounded by its own measurable electrical field. As the excitation process (depolarization) spreads (and precedes muscular contraction), there results complete electronegativity on the exterior of the cell membranes (end of depolarization). At this point there are no longer any dipoles present, and therefore there is no measurable electrical field. During the recovery phase of the heart muscle, metabolic changes occur. These bring about once more positive charges on the outside of the membrane. This, then, is the beginning of repolarization. Once more, dipoles exist and an electrical field is detectable. This process continues until the resting stage of the myocardium is achieved. By this time, the membranes of the muscle units have regained their impermeability and the heart muscle is ready for another cycle.

The simple dipoles and therefore the electrical fields that are so created during the period of depolarization (excitation) of the heart muscle may be represented in a graphic manner by means of arrows, which are termed vectors. These vectors (arrows) provide the following information: The length of the

arrow indicates magnitude or strength of the electrical field it represents; the position of the arrow's shaft represents the direction of the electrical field (dipole); and its tip or terminus points into positive polarity.

The excitation wave is initiated at the pacemaker or sinus node and spreads down through the atria to the AV node (Fig. 3). From this point it continues through the bundle, its branches, Purkinje's network, and the ventricular muscle mass. A great many diverse dipoles or vectors, which are different in direction, polarity, and magnitude, are created in both atria and ventricles. The electrical forces surrounding the heart are naturally three-dimensional or spatially oriented. Since the excitation stimulus affecting various parts of the heart muscle occurs sequentially and at different times, it follows that successive and

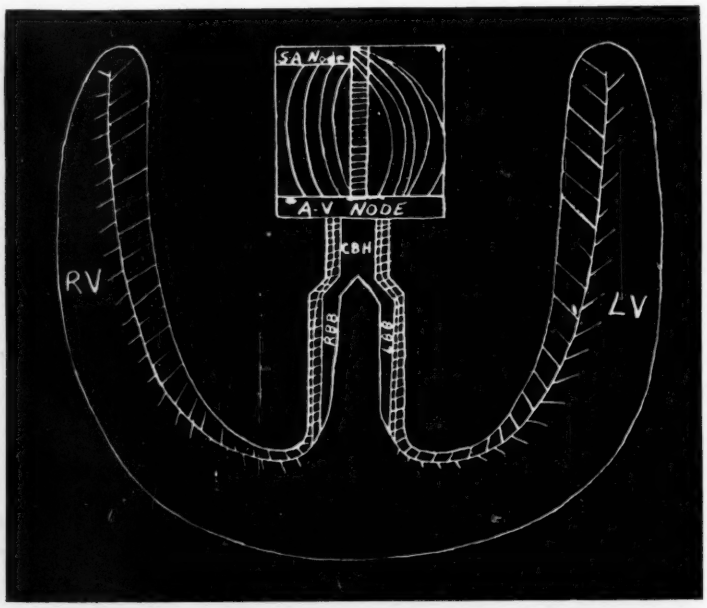

Fig. 3.—Spread of excitation wave. (From Grishman and Scherlis: Spatial Cardiography, Philadelphia, 1952, W. B. Saunders Company.)

diverse electric forces or vectors will thus be created. It also follows that, from the beginning to the completion of depolarization and repolarization, there develop countless vectors occurring one after the other or in sequence. At any particular instant of time it is possible to sum up geometrically and algebraically a large number of these vectors into a single instantaneous or resultant vector (Fig. 4). The diagrams in Fig. 4 indicate the vectorial forces which operate at three successive time intervals during ventricular contraction at *a*, *b*, and *c*. *A*, *B*, and *C* illustrate the resultant vectors of all these contributory electrical forces at any one given instant. This single resultant vector *A*, *B*, *C* describes the direction, magnitude, and sense or polarity of the electromotive field of the heart at a given instant. Each cardiac cycle is represented by a smaller number of diverse instantaneous resultant vectors whose point of origin is considered to be the electrical center of the heart represented

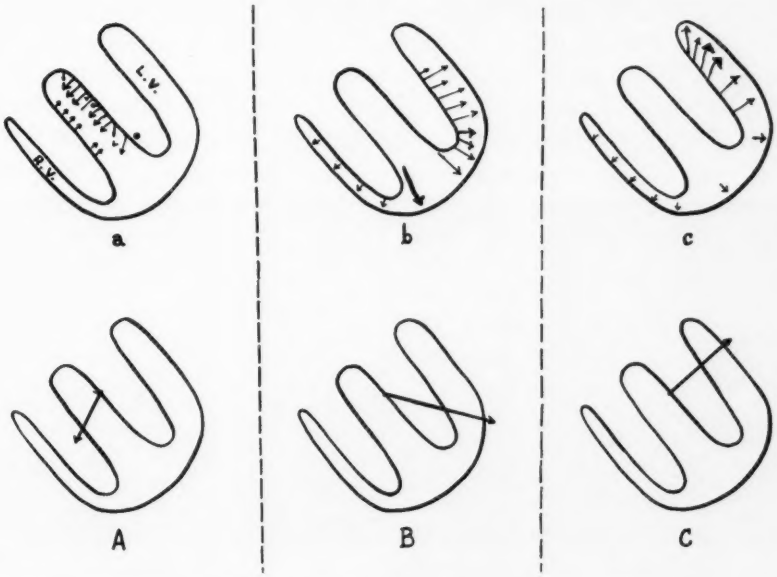

Fig. 4.—Coronal section of the heart. *LV*, Left ventricle; *RV*, right ventricle. *a*, *b*, and *c* depict the many forces operative in the heart at successive time intervals of depolarization. *A*, *B*, and *C* portray the resultant vectors at each specific time interval. A single vector represents the resultant direction, magnitude, and polarity of the many contributory individual forces at any given time. (From Grishman and Scherlis: Spatial Cardiography, Philadelphia, 1952, W. B. Saunders Company.)

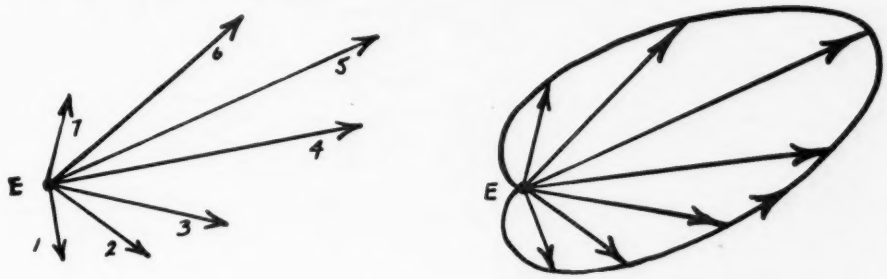

Fig. 5.—*A*, Diverse instantaneous vectors representing each cardiac cycle. *B*, Closed loop produced by connecting arrow tip. (From Grishman and Scherlis: Spatial Cardiography, Philadelphia, 1952, W. B. Saunders Company.)

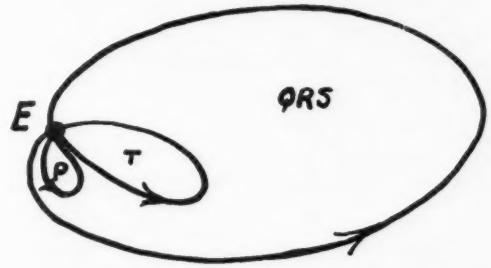

Fig. 6.—Three vectorcardiographic loops (see text). (From Grishman and Scherlis: Spatial Cardiography, Philadelphia, 1952, W. B. Saunders Company.)

schematically by point *E* (Fig. 5). From this point emanate in sequence of time the instantaneous vectors referred to above. A line connecting the arrow tips of these vectors produces a closed loop which, as may be seen in the reproduction, begins and ends at point *E* (Fig. 5). There are three such loops (Fig. 6). The largest represents ventricular excitation (depolarization) and is termed

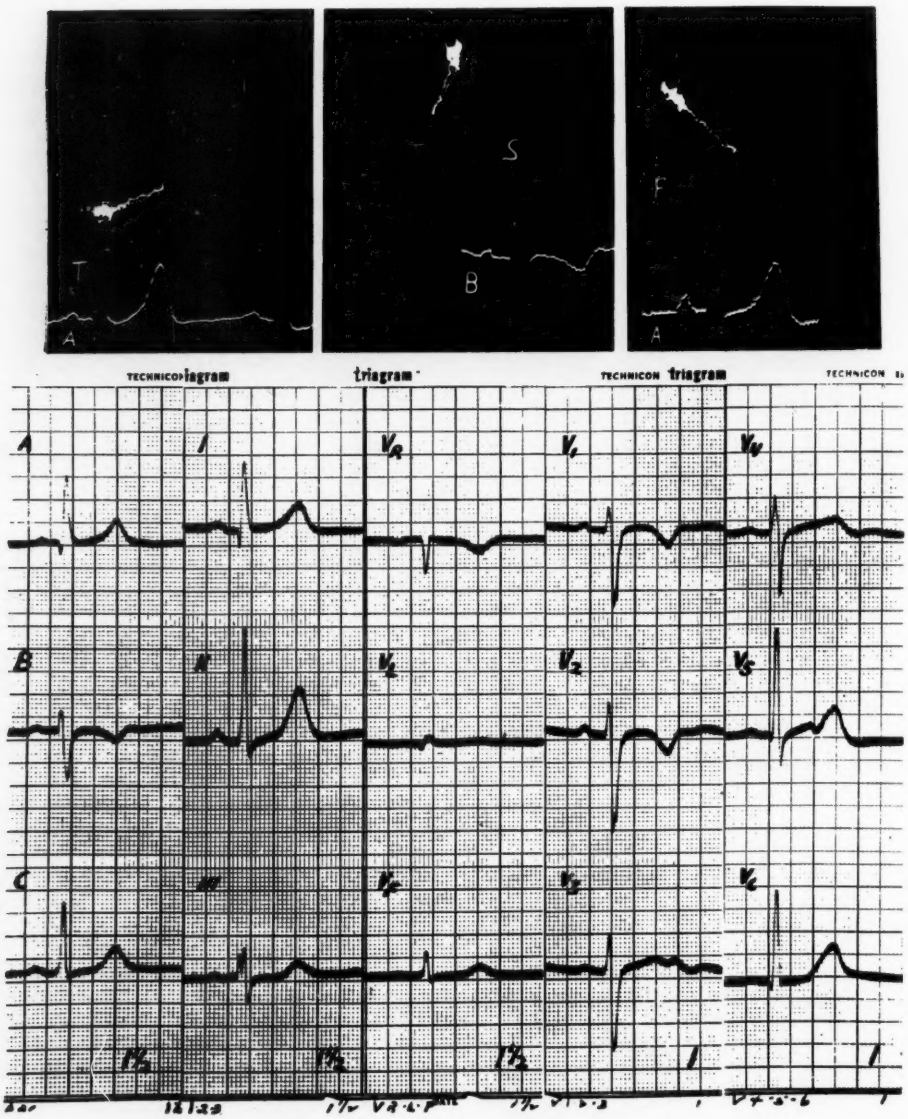

Fig. 7.—Normal vectorcardiogram and electrocardiogram of same subject. Horizontal (transverse), sagittal and frontal plane projections are seen from left to right. Below each loop is one of the bipolar component leads, *A*, *B*, or *C*.

The horizontal (T) QRS loop is inscribed in a counterclockwise direction and is oriented to the left and posteriorly. The sagittal (S) QRS loop, inscribed in a clockwise direction, reveals centrifugal anterior and inferior portions followed by centripetal posterior and superior portions. The QRS loop in the frontal plant (F) shows a counterclockwise rotation. The derived electrocardiogram is also normal. Standardization in noted in each set of simultaneous leads. The recording spread is twice normal (50 mm./sec.). (From Grishman and Scherlis: Spatial Cardiography, Philadelphia, 1952, W. B. Saunders Company.)

the QRS loop. It is analogous to the familiar QRS complex in the electrocardiogram. The smallest loop is the P loop. The intermediate loop is the T loop which corresponds to the T wave of the electrocardiogram and is produced during ventricular recovery (repolarization). Each loop consists of a series of interrupted lines produced electronically and shaped like teardrops or minnows, with the plump end facing into the direction of the inscription (Fig. 7). Each of these interruptions occurs every $\frac{1}{400}$ second. In this manner it is possible to determine both the speed and the direction of inscription of the loop.

The scanning of the vector loops includes attention to several factors which characterize the normal loops[25]: the orientation in space of the loop, the direction and speed of its inscription, its starting and finishing point, and the relationship in space between the P, QRS, and T loops.[26, 27]

RESULTS

A total of 177 patients with severe bronchial asthma are included in this study. There are 100 male patients and seventy-seven female patients. Table II shows the distribution of patients according to age groups. It is interesting, but

TABLE II. INCIDENCE OF PULMONARY COMPLICATIONS OF ASTHMA WITH REFERENCE TO AGE OF PATIENT

AGE	NUMBER OF PATIENTS					
	CHRONIC BRONCHITIS	EMPHYSEMA	FIBROSIS	BRONCHI-ECTASIS	PRESENCE OF ONE OR MORE COMPLICATIONS	TOTAL
4-10	—	—	—	—	—	2
10-20	—	—	—	—	—	7
20-40	13	7	5	2	15	52
40-60	48	31	23	6	48	88
60+	23	17	14	2	23	28
Total patients	84	55	42	10	86	177

not surprising, to note that pulmonary complications, such as chronic bronchitis, pulmonary emphysema, fibrosis, and bronchiectasis, occur in a much higher percentage of the older age group and in patients whose asthma is of long duration (Table III). The criteria for the diagnosis of these complications were as follows:

TABLE III. INCIDENCE OF PULMONARY COMPLICATIONS OF ASTHMA WITH RELATION TO DURATION OF DISEASE

DURATION (YEARS)	NUMBER OF PATIENTS					
	CHRONIC BRONCHITIS	EMPHYSEMA	FIBROSIS	BRONCHI-ECTASIS	PRESENCE OF ONE OR MORE COMPLICATIONS	TOTAL IN EACH GROUP
1-5	11	4	5	1	11	77
6-10	22	15	12	3	24	41
10+	51	36	25	6	51	59
Total patients	84	55	42	10	86	177

1. Chronic bronchitis was diagnosed in the presence of frequent periodic respiratory infections precipitated by changes in humidity and temperature. These patients have an early morning cough which is productive of scanty sputum or of a viscid, thick, purulent sputum. Bacteriologic study carried out in fourteen of these patients yielded pyogenic organisms—chiefly *Streptococcus hemolyticus, pneumococci, Micrococcus catarrhalis, Hemophilus influenzae,* etc. Six of the patients were bronchoscoped. Bronchiectasis was ruled out because bronchoscopic and bronchographic examinations were negative and because, for the most part, the cough was paroxysmal and recurrent. It is realized, of course, that bronchitis is a difficult diagnosis to make—one about which there may exist considerable difference of opinion among experts.

2. Pulmonary fibrosis and emphysema were diagnosed on the basis of physical findings which included mild to marked cyanosis and an increase in the anteroposterior diameter of the chest; chest roentgenograms showed that two patients had localized emphysema with bulla formation. In the patients with chronic obstructive pulmonary emphysema, there was widening of the intercostal spaces; the heart was vertical and small; the diaphragm was low, depressed, and flattened; bronchial markings were prominent; fluoroscopic examination of the chest revealed a relatively stationary diaphragm with restricted motion from inspiration to expiration and poor lung expansion; an increased translucency of the lung fields was demonstrable; and there was a definite decrease in timed vital capacity studies in twelve of our cases.

3. In the patients with bronchiectasis there was a copious mucopurulent sputum; roentgenograms of the chest indicated pronounced bronchial markings; and in three instances the bronchograms showed dilated, cylindrical, or sacular bronchial markings. In these last three patients bronchoscopic examination showed dilatation of the bronchi and inflammation of the bronchial mucosa, which was covered with purulent sputum.

Hypotension is demonstrable in fifty-eight patients, or in 32 per cent of the series. The significance of this rather common finding is not understood. It may possibly be related to autonomic nervous system influence.

Table IV indicates the electrocardiographic conclusions in complicated and uncomplicated asthma. The usual criteria for electrocardiographic diagnosis are employed. However, bradycardia, sinus arrhythmia, occasional premature contractions, and axis deviation are not considered as a basis for the diagnosis of abnormality. If we group together the probably abnormal with the unequivocally abnormal electrocardiograms, we find a total of twenty-nine (16.3 per cent) abnormal electrocardiograms in the entire group of patients. In the same group there are 148 normal and probably normal tracings. A detailed analysis indicates that in twelve of the twenty-nine abnormal electrocardiograms the abnormalities are due to unrelated, coincidental cardiac conditions,

such as old myocardial infarction, congestive heart failure, hypertension, rheumatic heart disease, and auricular fibrillation. Therefore, actually only seventeen patients (9.6 per cent) had abnormal electrocardiograms related to bronchial asthma. Of these seventeen tracings, nine show high P waves. All of these P waves are associated with clinical and radiologic evidence of pulmonary emphysema. The tracings showing high P waves were found, for the most part, in patients who were past 55 years of age and whose asthma was of more than five years' duration. In five of these nine cases there is corroborative evidence of cor pulmonale. This evidence consisted of a reduction of 30 to 50 per cent in vital capacity, with a corresponding increase in respiratory minute volume; an accentuation of the second pulmonic sound; enlargement of the right heart and prominence of the pulmonary artery and conus as seen in the roentgenogram of the heart; and, finally, suggestive electrocardiographic changes.

TABLE IV. ELECTROCARDIOGRAPHIC CHANGES IN COMPLICATED AND IN UNCOMPLICATED ASTHMA

	PULMONARY COMPLICATIONS IN ASTHMA						
ECG DIAGNOSIS	CHRONIC BRONCHITIS	PULMONARY EMPHYSEMA	PULMONARY FIBROSIS	BRONCHIECTASIS	ONE OR MORE COMPLICATIONS	PATIENTS WITH NO COMPLICATIONS	TOTAL
Normal	54	35	25	7	56	77	133
Abnormal	21	14	12	1	21	5	26
Probably normal	7	6	5	2	7	8	15
Probably abnormal	2	+	—	—	2	1	3
Total patients	84	55	42	10	86	91	177

In three instances, ST deflections and nonspecific T wave changes are seen in the chest leads, suggestive of possible myocardial anoxemia.

An analysis of the 177 vectorcardiograms reveals that a total of 138 (78 per cent) vectorcardiographic tracings show various degrees of abnormalities, the exact nature of which will be described later. This merely means that, in the light of our present understanding of vectorcardiographic interpretation, a sizable number of asthmatic patients present changes in the total electrical field of the heart. It is interesting to point out that this abnormality exists in ninety-eight out of 133 patients whose electrocardiogram is perfectly normal. Furthermore, as is to be expected, when the electrocardiogram is abnormal the vectorcardiogram also registers some change. This is illustrated by the fact that out of twenty-nine patients with abnormal electrocardiograms, twenty-seven also had abnormal vectorcardiograms.

Let us consider the relationship between the electrocardiographic and the vectorcardiographic findings. In classifying a spatial vectorcardiogram as abnormal, we took the following into consideration (Fig. 5):

1. QRS loop aberrations:

(a) Deviations from the normal QRS orientation which is posteriorly, to the left, and inferiorly.

2. Conduction delay of the:

(a) Right bundle branch type.

(b) Left bundle branch block variety.

(c) Nonspecific slowing of the QRS loop inscription.

3. Distinct posterior "pip" (promontory) oriented to the right, posteriorly, and superiorly.

4. Primary T loop changes without angular deviation:

(a) Symmetrically inscribed T forces.

5. Significant angular deviation (spatial QRS-T angle in two of three planes greater than 50 degrees) without change in the T loop.

6. Significant angular deviation with primary T loop change.

7. P loop abnormalities.

8. Abnormality in the direction of inscription of the QRS vector loops.

9. Presence of an ST vector.

Fig. 7 shows a photograph of a normal spatial vectorcardiogram as seen in the three planes marked *T* (horizontal or transverse) *S* (sagittal) and *F* (frontal). The time markings consist of interruptions which occur every $\frac{1}{400}$ second. The inscriptions are even. Physiologic slowing is manifested by closer spacing of the interrupted segments, both at the beginning and at the completion of the registration of the QRS loop. The large incidence of vectorcardiographic abnormalities indicates the high degree of sensitivity of the vectorcardiograph in recording more subtle electrical changes occurring in the heart.[28]

The vectorcardiogram shows marked rotation of the QRS loop posteriorly and to the left toward the anteroposterior axis in sixty-seven cases (32 per cent) (Table V A). Percentagewise, in proportion to the number of patients included in each disease duration group, 37 per cent whose asthma was of one to five years' duration, 40 per cent of the six- to ten-year group, and 46 per cent of the group whose asthma was of more than ten years' duration had rotation of this type (Table V B). The pulmonary complications occur, as may be seen in Table II, more frequently in older patients whose asthmatic condition is of

TABLE V A. RELATIONSHIP OF VECTORCARDIOGRAPHIC EVIDENCE OF POSTERIOR AND LEFTWARD ROTATION TO PULMONARY COMPLICATIONS

RS IN ALL V LEADS	CHRONIC BRONCHITIS	PULMONARY EMPHYSEMA	PULMONARY FIBROSIS	BRONCHIECTASIS	TOTAL
Rotation RVP LVP	36	26	18	6	67
Totals	36	26	18	6	67 (32%)

TABLE V B. VECTORCARDIOGRAPHIC EVIDENCE OF POSTERIOR AND LEFTWARD ROTATION TO DURATION OF DISEASE

DURATION OF ASTHMA	TOTAL NO. PATIENTS	PER CENT OF PATIENTS SHOWING THESE CHANGES
1-5	77	30
6-10	41	40
10+	59	46
Totals	177	

longer duration. As is well known, posterior displacement of QRS forces is found in older normal persons. However, these changes occur to a more marked degree in our asthmatic patients. Furthermore, unlike the presence of such alterations in normal older persons, our asthmatic patients also showed, as an accompaniment, marked ST vector and T loop changes. It may therefore be concluded that as the duration of the disease increases there is a greater proportion of patients displaying vectorcardiographic QRS loop rotation. Primary T loop changes and angular deviation are distributed as follows: eight (16 per cent) in the fifty-two patients comprising the 20- to 40-year-old group; twenty-six (28 per cent) in the eighty-eight patients of the 40- to 60-year-old group; thirteen (46 per cent) in the twenty-eight patients over 60 years of age. These changes (T loop) occur with the following frequency in relation to the duration of the patients' asthma (Table VI): twelve (15 per cent) of the seventy-seven patients in the one- to five-year duration group; twelve (30 per cent) of forty-one patients in the six- to ten-year duration group; and twenty-three (39 per cent) of fifty-nine patients in the group whose asthma is of more than ten years' duration. In summary, a total of forty-seven patients (26 per cent) out of the entire series of 177 present evidence of primary T loop changes and angular deviation. These findings are noted in an increasing number of older patients, percentagewise, and certainly in a larger proportion of the patients whose asthma is of longer duration. Twelve of these forty-seven abnormalities in the T loop were due to unrelated cardiac conditions, thus leaving a total of thirty-five (20 per cent) such vectorcardiographic aberrations. The significance of this finding is to be discussed later.

TABLE VI A. PRIMARY T LOOP CHANGES AND ANGULAR DEVIATION IN RELATION TO AGE OF PATIENT

AGE	NO. OF PATIENTS	PRIMARY T LOOP CHANGES AND ANGULAR DEVIATION
4-10	2	None
10-20	7	None
20-40	52	8 (16%)
40-60	88	26 (28%)
60+	28	13 (46%)
Totals	177	47 (26%)

TABLE VI B. PRIMARY T LOOP CHANGES AND ANGULAR DEVIATION IN RELATION TO DURATION OF DISEASE

DURATION OF DISEASE	NUMBER OF PATIENTS	PRIMARY T LOOP CHANGES AND ANGULAR DEVIATION
1-5	77	12 (15%)
6-10	41	12 (30%)
10+	59	23 (39%)
Totals	177	47 (26%)

Type I and Type II vectorcardiographic evidence of right ventricular preponderance, as seen here, is similar to that found in congenital heart disease, particularly in interatrial septal defect, as shown by one of us.[29] Type V is similar to what is seen occasionally in mitral stenosis and in postoperative pulmonary stenosis. There were two cases of Type I, one case of Type II, and

ten cases of Type V, making a total of thirteen such abnormalities. It appears that with an increase in the duration of the disease there occurs a higher incidence of Type V right ventricular preponderance. This pattern may possibly represent the presence of cor pulmonale which is not yet clinically detectable.

Fifty-one of fifty-eight patients with vectorcardiographic evidence of a posterior "pip" pattern had normal electrocardiograms. The exact meaning of the posterior "pip" pattern is not as yet clear. We suspect that it may signify an electrical change indicative of possible early right ventricular preponderance. The posterior "pip" pattern consists of a small terminal appendage or promontory oriented to the right posteriorly and superiorly.

Furthermore, ten (6 per cent) of the eleven patients who demonstrate vectorcardiographic evidence of right atrial preponderance (hypertrophy) have had the disease for more than six years. It does not occur in the remaining 166 cases. This abnormality most likely reflects long-standing pulmonary hypertension. Of eleven patients who show right atrial preponderance in the vectorcardiogram, only two show it in the electrocardiogram. This is characterized by the appearance of the P pulmonale pattern.

Only three out of 177 patients present frontal plane QRS loops that are frankly oriented in the frontal plane quadrant of so-called right axis deviation; thirty-one demonstrated a tendency toward a "vertical axis," but the QRS loops were not deviated sufficiently to result in a classical right axis deviation in the derived frontal plane leads. Interestingly enough, twenty-four patients have a frontal plane vectorcardiogram manifesting itself electrocardiographically as a marked left axis deviation. The majority of the group of patients studied show normal frontal plane vector forces and no axis deviation. In other words, it is quite apparent that changes in the frontal plane as reflected by axis deviation provide no specific information as to the effect of bronchial asthma on the electrical field of the heart. This is to be expected, since most of the important deviations of the cardiac electrical field are projected onto the all-important horizontal plane as tapped by the chest or V leads. Furthermore, it may be concluded that axis deviation, manifesting only frontal plane changes, is never to be considered pathognomonic of right or left ventricular hypertrophy. This principle is evidenced by the finding that of the thirteen patients who show undeniable vectorcardiographic evidence of right ventricular preponderance, none have frontal plane loops that fall into the right axis deviation quadrant.

DISCUSSION

Interesting information is gained from an examination of the data obtained from an analysis of the material available in this study of 177 cases of long-standing bronchial asthma.

Pulmonary emphysema and other chronic pulmonary complications develop in association with or as a result of long-standing untreated bronchial asthma. This type of pulmonary pathology is produced by the chronic, intermittent, prolonged obstruction to expiration with the consequent dilatation of the alveolar

ducts and bronchioles. There subsequently occurs considerable flattening of the walls of these structures, accompanied by tearing and even destruction of capillaries. This, in turn, results in diminution of and interference with alveolar circulation and increased pressure within the pulmonary arteries. Thus, an increased burden is added to the right ventricle, resulting in right ventricular hypertrophy and dilatation. Pulmonary hypertension and decreased vital capacity are associated with dyspnea, cyanosis, occasional hemoptysis, and clubbing of the fingers. In the more advanced cases we encounter findings characteristic of chronic cor pulmonale. That electrocardiographic changes occur in pulmonary emphysema is shown in a report by Wasserburger and coworkers,[30] who found a T-a wave depression of 0.5 mm. or more in 133 patients out of 1,920 cases of pulmonary disease. In 113 of the 133 cases showing cardiographic changes, there was evidence of moderate or severe pulmonary emphysema. It is difficult in many instances, however, to appraise electrocardiographically the presence of right or left ventricular hypertrophy, since the changes noted may indicate increased ventricular mass, dilatation, or increased intraventricular tension. Nor does one always find that changes in cardiac hemodynamics are reflected by alterations in the electrocardiogram.[31]

On the other hand, the spatial vectorcardiogram is of definite help in the determination of ventricular preponderance or hypertrophy, particularly of right-sided hypertrophy, for it deals with subtle changes in the sequential production of the single electrical field surrounding the heart.[29, 32]

Thirty-two per cent of the patients studied vectorcardiographically reveal displacement of the QRS loop in the horizontal plane, a projection posteriorly and to the left toward the anteroposterior axis. This has been termed[33] "the emphysema rotation pattern." It occurs mostly in patients whose asthma is of long duration and who have developed emphysema. These changes in the electrical field of the heart are probably due to disturbed anatomic relationships within the thoracic cavity and to altered respiratory mechanics. This finding is not inconsistent with the frequent finding of a posterior "pip" pattern in normal children with physiologic right ventricular preponderance. Some of the older asthmatic patients as well as normal children possessing this pattern have in common electrical preponderance of the right ventricle. Thirteen classic vectorcardiographic patterns of right ventricular preponderance appeared in our series. In most instances the vector alone supplied the important objective information that pulmonary hypertension existed. In several other patients, right atrial preponderance, not reflected in the electrocardiogram, is displayed vectorcardiographically. This again may be assumed to be another manifestation of increased pressure within the pulmonary circuit, resulting from long-standing bronchial asthma and its complications. In still another large group of patients we noted the presence of the so-called posterior "pip" pattern previously described as a normal variant in young persons. We observed this pattern as well in older patients with bronchial asthma. One may speculate that the posterior "pip" oriented to the right, posteriorly, and superiorly, may be indicative of electrical changes associated with early, subclinical pulmonary

hypertension with resultant right ventricular preponderance. The vectorcardiographic changes in the T loop reflect electrical abnormalities, about the presence of which there can be no doubt. They occur in 20 per cent of our patients suffering from asthma of long duration associated with pulmonary complications. Just what this implies clinically is not exactly clear. These alterations have been interpreted by some[34] as associated with coronary artery disease and myocardial anoxemia produced by various causes, of which pulmonary insufficiency might well be one; they also have been attributed to the influence of various nonspecific factors, such as stress and strain, autonomic nervous system imbalance, drugs, and early left ventricular preponderance. Most of our patients could well have been affected by some of these factors. It is well known that the severely asthmatic and emphysematous patient presents the clinical picture of pulmonary insufficiency associated to a variable degree with myocardial anoxemia—an objective evidence presented by the vectorcardiogram. These electrical changes are much less obvious and less frequently encountered in the electrocardiogram.. It is seen, therefore, that the vectorcardiogram offers valuable information concerning the changes in the total electrical field of the heart. It is a valuable supplement to electrocardiography.[35] It tells us the "why" of certain electrocardiographic aberrations[36] and offers a rational method of understanding the electrocardiogram rather than reliance on interpretation by memorization of patterns.[37]

The clinical cardiac examination, including x-ray evidence of cardiac enlargement, was singularly negative in all except five instances of cor pulmonale. Thus, on clinical grounds, there does not appear to be any evidence of cardiac involvement complicating bronchial asthma. This is to be expected, since a considerable degree of right ventricular hypertrophy may exist without increasing either the transverse diameter of the heart or the characteristics of the cardiac silhouette as viewed fluoroscopically.

SUMMARY AND CONCLUSION

1. One hundred seventy-seven patients with bronchial asthma of varying duration have been studied with reference to pulmonary and cardiac complications and electrocardiographic and vectorcardiographic changes.

2. Pulmonary emphysema and other pulmonary complications occur in a much higher percentage of patients whose inadequately treated asthma is of long duration.

3. Of the total group of patients, 9.6 per cent showed some electrocardiographic alterations specifically related to bronchial asthma.

4. There was a total of 138 (78 per cent) abnormal vectorcardiograms in our series of 177 cases.

5. Sixty-six per cent of the patients with normal electrocardiograms in this group showed vectorcardiographic abnormalities.

6. Thirty-two per cent of our patients had hypotension.

7. The vectorcardiographic abnormalities consisted of the following:

(a) Posterior and leftward "rotation" of the QRS loop in 32 per cent.

(b) Characteristic patterns of right ventricular preponderance, including the heretofore unemphasized Type V pattern, in 6 per cent.

(c) Right atrial preponderance in 6 per cent.

(d) Posterior "pip" pattern in 34 per cent.

(e) Primary T loop changes, with or without angular deviation, in 20 per cent.

8. These vectorcardiographic changes present unmistakable objective evidence of alterations in the electrical field of the heart, but as yet one cannot be dogmatic about the clinical interpretation of these alterations. They may well reflect the presence of pulmonary hypertension, myocardial anoxemia, and certain other influences.

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REFERENCES

1. Kahn, M. H.: The Electrocardiogram in Bronchial Asthma, Am. J. M. Sc. **173**: 555, 1927.
2. Alexander, H. L., Luten, D., and Kountz, W. B.: The Effects on the Heart of Long Standing Bronchial Asthma, J. A. M. A. **8**: 882, 1927.
3. Unger, L.: Heart in Bronchial Asthma; Electrocardiographic Study of Seventy-Four Cases, J. ALLERGY **2**: 17, 1930.
4. Criep, Leo H.: The Effect of Bronchial Asthma on the Circulation, Arch Int. Med. **49**: 241, 1932.
5. Wadsworth, G. H., and Brown, C. H.: Serum Reactions Complicated by Acute Carditis, J. Pediat. **17**: 801, 1940.
6. Binder, M. J., Gunderson, H. J., Cannon, J., and Rosove, L.: Electrocardiographic Changes Associated With Allergic Reactions to Penicillin, Am. Heart J. **40**: 940, 1950.
7. Lilienfeld, A., Hockstern, E., and Weiss, W.: Acute Myocarditis With Bundle Branch Block Due to Sulfonamide Sensitivity, Circulation **1**: 1060, 1950.
8. Foster, R. F., and Layman, J. D.: Generalized Urticaria With Electrocardiographic Changes Simulating Myocardial Infarction, J. A. M. A. **148**: 203, 1952.
9. Neustadt, D. H.: Transient Electrocardiographic Changes Simulating an Acute Myocarditis in Serum Sickness, Ann. Int. Med. **391**: 126, 1953.
10. Goldman, M. J., and Lau, F. Y. K.: Acute Pericarditis Associated With Serum Sickness, New England J. Med. **250**: 278, 1954.
11. Contro, S., and Moud, E.: The Electrocardiogram in Hypersensitivity Reactions, Am. Heart J. **52**: 510, 1956.
12. Rosenfeld, I., Silverblatt, M. L., and Grishman, A.: Allergic Shock in Humans: Report of Two Cases With Electrocardiographic Findings, Am. Heart J. **53**: 463, 1957.
13. Glotzer, S.: Electrocardiographic Changes During Sensitivity Reaction to Penicillin, Am. Heart J. **47**: 300, 1954.
14. Colton, W. A., and Ziskin, T.: The Heart in Bronchial Asthma, J. ALLERGY **8**: 347, 1937.
15. Harkavy, J., and Romanoff, A.: Electrocardiographic Changes in Bronchial Asthma and Their Significance, J. ALLERGY **12**: 405, 1941.
16. Urbach, E.: Allergy, New York, 1943, Grune & Stratton, Inc., p. 696.
17. Schiller, I. W., Colmes, A., and Davis, D.: The Occurrence of Cor Pulmonale in Cases of Bronchial Asthma, New England J. Med. **228**: 113, 1943.
18. Rom, J., and Jend, W.: Electrocardiographic Studies in Bronchial Asthma, J. ALLERGY **20**: 428, 1949.
19. Criep, Leo H.: Electrocardiographic Studies of the Effect of Anaphylaxis on the Cardiac Mechanism, Arch. Int. Med. **48**: 1098, 1931.
20. Criep, Leo H., and Riley, W. K.: Electrocardiographic Studies of the Effect of Histamine on the Cardiac Mechanism, Am. Heart J. **41**: 423, 1951.
21. Mikalicich, G.: Electrocardiographic Changes in Experimental Anaphylactic Reactions, J. ALLERGY **22**: 249, 1951.
22. Editorial: Obstructive Pulmonary Emphysema, J. ALLERGY **29**: 375, 1958.

23. Grishman, A., and Scherlis, L.: Spatial Vectorcardiography, Philadelphia, 1952, W. B. Saunders Company.
24. Grishman, A., Borun, E. R., and Jaffe, H. L.: Spatial Vectorcardiography: Technique for the Simultaneous Recording of the Frontal, Saggital and Horizontal Projections, Am. Heart J. **41**: 483, 1951.
25. Scherlis, L., Lasser, R. P., and Grishman, A.: Spatial Vectorcardiography: the Normal Vectorcardiogram, Am. Heart J. **42**: 235, 1951.
26. Milnor, W. R.: The Normal Vectorcardiogram and a System for the Classification of Vectorcardiographic Abnormalities, Circulation **16**: 95, 1957.
27. Young, E., Wolff, L., and Chatfield, J.: The Normal Vectorcardiogram, Am. Heart J. **51**: 713, 1956.
28. Allenstein, B. J.: Direct Spatial Vectorcardiography; Clinical Application, Dis. Chest **30**: 359, 1956.
29. Silverblatt, M. L., Rosenfeld, I., Grishman, A., and Donoso, E.: The Vectorcardiogram and Electrocardiogram in Interatrial Septal Defect, Am. Heart J. **53**: 380, 1957.
30. Wasserburger, R. H., Ward, V. G., Cullen, R. E., Rasmussen, H. K., and Juhl, J. H.: The T-a Wave of the Adult Electrocardiogram: An Expression of Pulmonary Emphysema, Am. Heart J. **54**: 875, 1957.
31. Editorial: Clinical Electrocardiography at Crossroads, Am. J. Med. **24**: 831, 1958.
32. Sapin, S. O., Donoso, E., Braunwald, E., and Grishman, A.: Spatial Vectorcardiography in the Diagnosis of Congenital Cardiac Malformations, Pediatrics **17**: 93, 1956.
33. Stern, E., and Tenney, S.: Correlation of the Spatial Vectorcardiogram and the Electrocardiogram in Right Ventricular Hypertrophy, Am. Heart J. **51**: 53, 1956.
34. Karni, H.: The Ts E Loop in Hypercholesterolemia, Am. Heart J. **54**: 269, 1957.
35. Duchosal, P. W.: The Information Derived From Vectorcardiograph; Supplements Obtained From the Electrocardiogram, Am. Heart J. **53**: 1, 1958.
36. Grant, R. P., and Estes, E. H., Jr.: Spatial Vector Electrocardiography, New York, 1951, The Blakiston Company.
37. Kornbluth, A. W., and Allenstein, B. J.: The Normal Direct Spatial Vectorcardiogram, Am. Heart J. **54**: 396, 1957.

Bigelow Apartments.

THE USE OF THE VALSALVA TEST IN THE DIFFERENTIAL DIAGNOSIS OF BRONCHIAL ASTHMA

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ORDINARILY, little difficulty is encountered in making a diagnosis of bronchial asthma. The typical physical finding of sibilant and sonorous râles throughout both lung fields with prolongation of the expiratory phase, supported by a history of allergy and of recurring attacks, is usually ample and conclusive evidence for diagnosis and an indication for specific treatment. On occasion, however, there is an atypical, borderline case in which differentiation between bronchial asthma and cardiac asthma is not distinct and the exact diagnosis can be made only after detailed and lengthy investigation.

Another preplexing aspect of the differential diagnosis which confronts the allergist treating chronic bronchial asthma may be encountered in a patient 40 to 60 years of age who has suddenly or gradually developed resistance to his usual allergic management. Ordinary measures which previously were effective now fail to prevent the recurrence of attacks. One complicating factor frequently responsible for these atypical responses to previously effective therapy is the development of incipient left heart failure. Clinical improvement following the administration of diuretics, digitalis, and other cardiac supportives will usually clarify the clinical picture, but often these measures are too time consuming. Other diagnostic aids, such as vital capacity, venous pressure determination, circulation time, etc., often are not readily available and frequently are unreliable and inconclusive. It must also be remembered that the therapeutic response to certain drugs is equally effective in both conditions.

Recently a simple bedside procedure has been investigated, described, and popularized for the diagnosis of left heart failure.[1] This procedure makes use of the well-known Valsalva maneuver as an indication of early and incipient left heart failure. The accuracy of this test has been correlated and confirmed by using direct brachial arterial tracings simultaneously recorded through an indwelling Riley needle connected to a Sanborn electromanometer.[1a] It would appear that such a simple, practical, and easily performed test would be of distinct value in differentiating cardiac asthma from bronchospasm due to allergy or other causes.

The Valsalva test involves the recording of blood pressure variations during the performance of the Valsalva maneuver. It would be best to review briefly

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the simple materials needed, the method of application, and the interpretation of normal and abnormal responses to the test. After very little practice the examiner will find that the Valsalva test is easily performed and readily interpreted. The materials used are few and readily available. These include (1) an aneroid sphygmomanometer gauge attached to (2) a short piece of rubber tubing tipped with (3) a mouthpiece (an inverted ear speculum will serve nicely), (4) a sphygmomanometer (aneroid or mercury with usual cuff), and (5) a stethoscope.

The patient's basal blood pressure is recorded following a short period of rest in the supine position. Upon a given signal by the examiner, the patient expires forcibly into the mouthpiece for ten seconds with immediate sufficient force to reach and maintain a reading on the gauge. At the same time, the examiner, having set his cuff blood pressure reading at 30 to 40 mm. Hg above the previously established resting systolic blood pressure, determines easily by auscultation the existence of systolic beats and their changing levels during this strain phase. The patient is then instructed to resume normal respiration, at which time the cuff pressure is again raised to 20 to 30 mm. Hg above the basal blood pressure level, and the examiner notes once again the presence or absence of systolic beats during this resting stage.

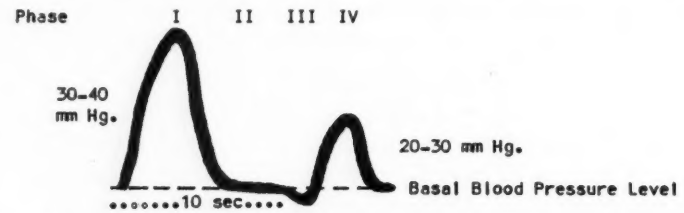

Fig. 1.—Normal Valsalva test pattern showing prominent rise in systolic blood pressure during strain phase (phase I) and overshoot phase (phase IV).

For interpretation of the test, four phases are noted. In the negative or normal Valsalva test response (Fig. 1), phase I consists of an initial rise in the systolic pressure to 30 to 40 mm. Hg above basal pressure and is referred to as the "strain phase." Phase II reflects a gradual fall in the systolic pressure during the remainder of the ten-second period. Phase III reflects a further fall in the systolic pressure when normal respiration is resumed. Phase IV reveals an increase in the systolic blood pressure 20 to 30 mm. Hg over the normal resting blood pressure within five seconds after resumption of normal respiration. This is known as the "overshoot" or "rebound phase."

Familiarization with the procedure by the examiner will soon dispel error in technique, but several precautions to prevent false positive reactions should be taken. The initiation of the strain phase should be rapid and forceful enough to bring the pressure immediately to 40 mm. Hg, or the first phase will not occur. Care should also be exercised in preventing the patient from expiring slowly when he is told to resume normal respiration, as this will prevent the normal rebound in phase IV.

The abnormal or positive test pattern seen in patients with congestive heart failure is remarkable in its striking difference (Fig. 2). Here the strain phase is definitely prolonged, with retention of the increase in pulse pressure depending upon the severity of congestive heart failure. Also, after resumption of normal respiration, there is an absence of the rebound or overshoot phase.

Another interesting and consistent abnormal response pattern confirming the results obtained by Irvin[2] and by Mills and Kattus[3] in cases of uncomplicated pulmonary emphysema revealed a sharp fall below the basal level immediately after the onset of the strain phase. This continued until after resumption of normal respiration, when the usual overshoot then occurred (Fig. 3).

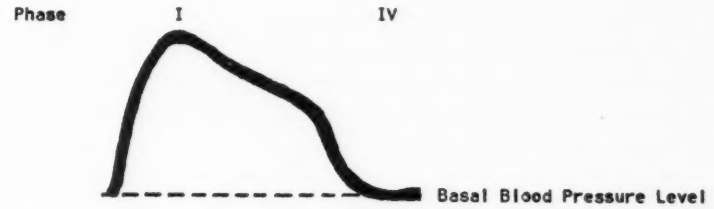

Fig. 2.—Abnormal Valsalva test pattern showing continued persistence of strain phase (phase I) and complete absence of overshoot (phase IV) as seen in left ventricular failure, or cardiac asthma.

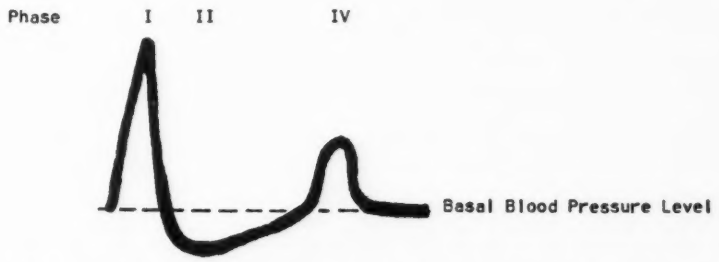

Fig. 3.—Typical Valsalva test response in uncomplicated pulmonary emphysema revealing extremely short strain phase (phase I), very low phase II, and prominent overshoot (phase IV).

This study concerns the use of the Valsalva test as a singular diagnostic means to differentiate bronchial asthma from cardiac asthma (shortness of breath with wheezing due to pulmonary congestion). Forty-nine cases were studied. Included within this group were twelve patients who had left heart failure with pulmonary congestion, confirmed by the presence of two or more of the following accepted criteria of symptoms and signs: increasing dyspnea on exertion, orthopnea, paroxysmal nocturnal dyspnea, moist râles in the bases of the lungs, prolonged circulation time, and favorable response to digitalis or diuretics. The etiology of their heart disease varied and included coronary artery disease, arteriosclerosis, hypertension, and (in one case) arteriosclerosis with diabetes. The remaining thirty-seven patients had various stages of known bronchial asthma. Of these, two patients were known to have chronic pulmonary emphysema.

In each of the twelve cases with various degrees of congestive heart failure the Valsalva test was positive and revealed the typical pattern of a prolonged strain phase and absence of overshoot as shown in Fig. 2. Thirty-five patients with bronchial asthma showed the previously described rapidly rising and falling strain phase followed by an overshoot during the fourth phase (Fig. 1). Two patients who had bronchial asthma complicated by well-marked pulmonary emphysema showed exaggeration of the normal response as depicted in Fig. 3.

Early in this study two false positive results were obtained in testing uncomplicated bronchial asthma; this was caused by an improper technique. Several repeat tests performed later revealed typically normal pattern responses to the Valsalva test. In addition, an attempt was made to perform the Valsalva test on seven patients while they were in severe stages of acute asthma. Because of the technical difficulties experienced by these patients in their attempts to produce or maintain the strain phase, many bizarre patterns were obtained and it became necessary to discard these results. In each of these cases, however, a normal test pattern resulted when the acute state of asthma had lessened and the patient could be more cooperative.

The following cases illustrate the value of this procedure in differentiating bronchial asthma from cardiac asthma.

CASE 1.—The patient, a 62-year-old man, had suffered from chronic bronchial asthma for the past twelve years. Marked skin reactions to pollens had been obtained, and good control had been maintained with specific antigen therapy plus the usual antiasthmatic aids. Since coming to California nine months earlier, the patient had been having only mild and infrequent asthma. When seen, he presented moderately severe asthma of five days' duration; this was more severe at night and was not relieved by the usual supportive medication. At times the patient would awake from a sound sleep with an attack which would be partially relieved by his sitting up in bed. Examination of the chest revealed numerous sonorous and sibilant râles throughout both lung fields and an occasional moist râle in the left base. Because of the possibility of the existence of a complicating factor, perhaps congestive heart failure suggested by the paroxysmal nocturnal dyspnea, the Valsalva test was performed. This resulted in a typical normal test pattern. Specific allergic treatment was then reinstituted without delay, and the patient subsequently responded with ultimate complete relief.

CASE 2.—This patient, a man 62 years of age, had suffered attacks of bronchial asthma since childhood. The attacks gradually lessened in frequency and intensity until after the patient reached the age of 35, when severe attacks of wheezing would occur following any infection of the upper respiratory tract. One year prior to our seeing him, at Christmas time and again following a severe upper respiratory infection, the patient had intractable asthma which required hospitalization for eventual relief. After that attacks of wheezing, usually mild and sporadic, continued until about three weeks before we saw the patient, when there was aggravation of symptoms. The attacks rapidly became severe and frequently occurred at night, making it necessary for the patient to sit up in a chair. Antiasthmatic medication produced little to no relief. The attacks were not as severe during the day, and the patient was able to go about his work as a salesman. When first seen, he was only mildly dyspneic and presented no startling physical findings except for a few scattered sonorous râles throughout both lung fields. The recent lack of favorable response to previously effective medication suggested the presence of some complicating factor. The Valsalva test was then performed and revealed the typically abnormal pattern response seen in congestive heart failure. Cardiac measures were instituted without further delay and resulted in marked clinical improvement and obvious relief of asthma.

SUMMARY AND CONCLUSIONS

The Valsalva maneuver was applied as a test of congestive heart failure in a series of thirty-five patients with uncomplicated bronchial asthma due to allergic factors, twelve patients with wheezing dyspnea due to various degrees of frank, early, and incipient left heart failure, and two patients with wheezing and shortness of breath associated with pulmonary emphysema.

In uncomplicated pulmonary emphysema there is an exaggeration of the normal response, with an early rapid fall after an initial rise in the systolic blood pressure below the basal blood pressure level and a prominent secondary rise in the systolic blood pressure during the resting stage.

In this study, in all cases of bronchial asthma due to allergic factors where no cardiac involvement existed the test was negative. There was an initial rise of systolic blood pressure during the strain phase followed by a gradual reduction and a secondary increase. In twelve cases of mild to moderate congestive heart failure the test was positive, with prolongation of the initial rise during the strain phase and absence of the secondary rebound or overshoot. In two cases of pulmonary emphysema without evidence of left heart failure there was the typical exaggerated normal or negative response.

It is concluded that the Valsalva test is an effective, easily performed, simple bedside or office test for the differential diagnosis of bronchial asthma.

REFERENCES

1. (a) Knowles, J. H., Gorlin, R., and Storey, C. F.: Clinical Test for Pulmonary Congestion With Use of the Valsalva Maneuver, J. A. M. A. **160**: 44-48, 1956.
   (b) Gorlin, R., Knowles, J. H., and Storey, C. F.: Valsalva Maneuver as a Test of Cardiac Function: Pathologic Physiology and Clinical Significance, Am. J. Med. **22**: 197-212, 1957.
   (c) Ard, R. W., and Twining, R. H.: Evaluation of Valsalva Test in Bedside Diagnosis of Dyspnea, Am. J. M. Sc. **234**: 403-412, 1957.
   (d) Goldberg, H., Elisberg, E. I., and Katz, L. N.: Effects of Valsalva-like Maneuver Upon Circulation in Normal Individuals With Mitral Stenosis, Circulation **5**: 38-47, 1952.
   (e) Price, H. L., and Conner, E. H.: Certain Aspects of Hemodynamic Response to Valsalva Maneuver, J. Appl. Physiol. **5**: 449-456, 1953.
   (f) Greene, D. G., and Bunnell, I. L.: Circulatory Response to Valsalva Maneuver of Patients With Mitral Stenosis With and Without Autonomic Blockade, Circulation **8**: 264-268, 1953.
   (g) Hamilton, W. F., Woodbury, R. A., and Harper, H. T., Jr.: Arterial Cerebrospinal and Venous Pressures in Man During Cough and Strain, Am. J. Physiol. **141**: 42-50, 1944.
   (h) Sarnoff, S. J., Hardenbergh, E., and Whittenberger, J. L.: Mechanism of the Arterial Pressure Response to the Valsalva Test: The Basis for Its Use as an Indicator of the Intactness of the Sympathetic Outflow, Am. J. Physiol. **154**: 316-327, 1948.
   (i) Elisberg, E., Singian, E., Miller, G., and Katz, L. N.: The Effect of the Valsalva Maneuver on the Circulation. III. The Influence of Heart Disease on the Expected Poststraining Overshoot, Circulation **7**: 880-889, 1953.
   (j) Sharpey-Schafer, E. P.: Effects of Valsalva's Manœuvre on Normal and Failing Circulation, Brit. M. J. **1**: 693-695, 1955.
   (k) Stucki, P., Hatcher, J. D., Judson, W. E., and Wilkins, R. W.: Studies of Circulation Time During Valsalva Test in Normal Subjects and in Patient's With Congestive Heart Failure, Circulation **11**: 900-908, 1955.
2. Irvin, C. W.: Valsalva Maneuver as a Diagnostic Aid, J. A. M. A. **170**: 787-791, 1959.
3. Mills, H., and Kattus, A. A., Jr.: Emphysema Response to Forced Straining (Valsalva's Maneuver), Circulation **17**: 65-71, 1958.

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DEMONSTRATION OF ANTIBODIES IN THE SERA OF GRASS-SENSITIVE PERSONS BY THE BIS-DIAZOTIZED-BENZIDINE HEMAGGLUTINATION TECHNIQUE

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INTRODUCTION

In the past, demonstration of antibodies in the skin and in the sera of allergic patients has been limited to in vivo methods, such as the direct skin test and the passive transfer technique of Prausnitz-Küstner.[1] Both these methods are highly sensitive. The latter method enables one to detect minute amounts of antibody lower than 0.01 μg.[2] Because of the inherently biologic nature of these techniques, however, and particularly since the reactions elicited by the passive transfer test vary considerably in different subjects and even in different skin areas of the same subject the results obtained are difficult to quantitate. In addition, there is the as yet unavoidable danger of transferring serum homologous hepatitis to the test subject.

Consequently, a specific and sensitive in vitro test for the detection of antibodies in allergic sera has been sought for almost four decades. Unfortunately most of the commonly used immunologic techniques (for example, precipitation,[3-5] complement fixation,[6-9] Dale-Schultz test,[10, 11] and passive cutaneous anaphylaxis[12]), when applied to allergic sera, have led either to negative or equivocal results. The most promising indications for the feasibility of a reliable in vitro test were obtained with various agglutination techniques. In these methods, in general, some bulky carriers, such as collodion particles[13] or erythrocytes,[14] are coated with the antigen and these particles are aggregated in the presence of specific antibodies.

Kallós[15] used collodion particles "sensitized" with wheat or rye grass pollen extracts. Orlans, Rubinstein, and Marrack[5] applied Boyden's tanned-cell technique[14] to sera from grass-sensitive patients. The sensitivity of this technique with grass allergens was rather low, as evidenced by their results. Thus, only eight of twenty-two sera from untreated grass-sensitive persons gave positive titers, ranging from 1:6 to 1:96, and twenty-two of twenty-four sera

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from treated persons gave positive reactions, ten of which had titers over 1:100. Feinberg and associates[16] employed the same method with the sera of untreated and treated ragweed-sensitive patients and found positive reactions in 21 per cent and 67 per cent of their tests, respectively. Subsequently, however, these workers[17] reported that ragweed antigens were desorbed from the tanned cells and that consequently the liberated antigen(s) acted as inhibitor, diminishing the titer of strong sera and probably suppressing completely the reaction with weaker sera. Similar complications may well arise in the collodion particle method, as used by Kallós, since in this technique the antigen(s) are attached to collodion by nonspecific adsorption forces.

To overcome this limitation of the tanned-cell technique, particularly with antigens of low molecular weight, Sehon and colleagues[18] have recently coupled ragweed pollen constituents via stable covalent azo bonds to erythrocytes. Bis-diazotized benzidine was used as coupling agent and the method was similar to that developed by Pressman and associates[19] and modified by Stavitsky and Arquilla.[20] Using this method, Gordon and co-workers[21] were able to demonstrate antibodies in all sera of a group of forty ragweed-sensitive patients and proved this method to be highly specific and sensitive.

The purpose of the present study was to adapt the BDB hemagglutination technique for the detection of antibodies in sera of hay fever patients sensitive to the common American grasses and for establishing antigenic and allergenic relationships among the pollen constituents of these grasses. For standardization of the method, rabbit antisera to six grasses (timothy, June, orchard, redtop, sweet vernal, and Bermuda) were used.

MATERIALS AND METHODS

Antigens.—Glycerin-saline extracts of the pollens of timothy grass *(Phleum pratense)*, June grass *(Poa pratensis)*, orchard grass* *(Dactylis glomerata)*, redtop grass *(Agrostis alba)*, sweet vernal grass *(Anthoxanthum odoratum)*, and Bermuda grass *(Cynodon dactylon)* were used.† Each pollen extract was emulsified with an equal volume of complete Freund's adjuvant obtained from Difco Laboratories and the emulsion was used for immunization. The concentrations of the extracts used (expressed in terms of dry weights of the glycerin-saline extractable pollen constituents) were as follows: 11.35 mg./ml. for timothy, 5.18 mg./ml. for June, 6.24 mg./ml. for orchard, 6.21 mg./ml. for redtop, 5.33 mg./ml. for sweet vernal, and 5.21 mg./ml. for Bermuda.

Rabbit Antisera.—Six groups of five rabbits each were immunized with each of the six different grass pollen extracts according to the following schedules: Two rabbits received three weekly injections of 0.6 ml. of the antigen emulsion into the footpads. Two other rabbits received three weekly doses of 0.6 ml. of the emulsion intramuscularly. The fifth rabbit received both

*Known in Great Britain as cocksfoot grass.

†These extracts were most generously provided by Dr. Edward L. Foubert, Jr., Hollister-Stier Laboratories, Spokane, Washington.

types of inoculation over a period of three weeks, that is, 1.2 ml. per week. The sera of these animals were tested for antibodies by the "ring test" one to three weeks after the last footpad injection and three to six weeks after the last intramuscular inoculation. When precipitating antibodies were detected, 60 ml. of blood was collected from the marginal ear vein of the appropriate rabbit.

All animals were allowed to rest for two months and were then given a second course of immunization consisting of one intraperitoneal and two intravenous injections of 0.2 ml. of the glycerin-saline extract of grass pollen on successive days. This series of injections was repeated one week later. Several of the rabbits developed anaphylactic shock on receiving a booster dose of antigen. Therefore, it was decided that the last intravenous injections should be preceded by an intravenous dose of 0.04 mg./kg. Cycloheptidine* just prior to the antigen injection. No subsequent anaphylactic shocks were noted.

All sera were heated at 56° C. for thirty minutes to inactivate complement and were stored in a deep freeze at –20° C. until used.

Allergic Human Sera.—Sera were collected from thirteen untreated and seventeen treated patients† with grass pollen allergies at the Royal Victoria Hospital Allergy Clinic. The sera were "decomplemented" by incubation with zymosan for one hour at 37° C. or overnight with a thrice-washed precipitate of BSA-anti-BSA complexes in order to prevent hemolysis of erythrocytes during the hemagglutination test.

Hemagglutination Test.—

Erythrocytes: Rabbit erythrocytes were used in the hemagglutination technique. For this purpose, blood was collected from the marginal ear vein or by cardiac puncture into an equal volume of Alsever's solution[22] from rabbits which had not been previously bled or which had been rested for at least six weeks after an earlier bleeding. If the erythrocytes were obtained from rabbits bled within a shorter interval, false positive hemagglutination occurred. For storage of erythrocytes, the blood in the Alsever's solution was kept at 4° C. for as long as ten days.

Diluent: The serum used for diluent was separated from the blood of the same rabbit from which the erythrocytes were obtained. After inactivation of complement by heating at 56° C. for thirty minutes, it was diluted 100 fold with phosphate buffer at pH 7.3.‡

Bis-diazotized-benzidine: This was prepared according to the method used previously in this laboratory.[21] Before the antigen was coupled to erythrocytes, 1 ml. of frozen BDB stored in a vial was melted in the palm of the hand and added to 15 ml. of cold phosphate buffer at pH 7.3.‡ This solution will be

*Cycloheptidine is an antihistamine and antiserotonin preparation which was kindly supplied by Merck Sharpe & Dohme, Division of Merck & Co., Inc., Philadelphia, Pennsylvania.

†Sera obtained from patients who received hyposensitization treatment of grass pollen extracts will be referred to hereafter as treated sera. Untreated sera were collected from patients who never received injection therapy.

‡The phosphate buffer was prepared by mixing 215 ml. of 0.15 M disodium acid phosphate with 49 ml. of 0.15 M potassium dihydrophosphate.

referred to hereafter as BDB-phosphate solution. This must be used as soon as possible because it deteriorates quickly, forming a brown solution.

Sensitization of the erythrocytes: The rabbit red blood cells were washed three times with cold physiologic saline and the packed cells were resuspended in an equal volume of saline. The optimal quantity of grass pollen extract was then placed into a 15 ml. centrifuge tube and mixed with 0.1 ml. of 50 per cent red cell suspension. Finally, the optimal amount of BDB-phosphate (freshly prepared) was added and the mixture inverted several times to ensure even mixing. The reaction was allowed to proceed at room temperature* for fifteen minutes with occasional stirring. "Sensitized" cells were separated by centrifugation, and the brownish supernatant was discarded. The cells were washed with 3.5 ml. of diluent and recentrifuged. Finally the cells were resuspended in diluent to a final volume of 2.5 ml. This will be referred to as sensitized cell suspension.

Standardization of the method: To establish the optimal ratio of each batch of BDB for each of the pollen extracts, the following procedure was used. With a constant amount of erythrocytes, (1) the BDB-phosphate volume was varied, keeping the antigen concentration constant, and (2) the antigen concentration was varied and the BDB-phosphate volume was kept constant. In addition, two controls were used: each batch of sensitized cells was tested (1) with normal rabbit serum and (2) with the appropriate rabbit antigrass serum. The proportions of BDB-phosphate solution to grass pollen extract were considered optimal when the highest titers were obtained with immune sera and when no reaction ensued with the normal sera.

If insufficient antigen was used, the erythrocytes were coupled to each other by BDB and gave false positive patterns in the diluent. On the other hand, if too much antigen was used, the sensitivity of the method was lowered. Presumably, the antigen molecules were coupled preferentially to each other by the BDB and were not available for attachment to erythrocytes. For optimal sensitization, the following amounts of materials were used:

Cells	*BDB-phosphate*	*Glycerin-saline extracts*†
0.1 ml.	0.5 ml.	0.06 to 0.10 ml. timothy, orchard, redtop, June grass
0.1 ml.	0.5 ml.	0.10 to 0.13 ml. Bermuda grass
0.1 ml.	0.5 ml.	0.15 to 0.17 ml. sweet vernal

Glycerin itself, which is present in the pollen extracts, had no effect on the sensitization. When the antigen was replaced by glycerin in the sensitization procedure, the cells formed a negative pattern with the antisera used.

*A room temperature of 22 to 26° C. was ideal, but with temperatures over 28° C. rapid deterioration of the BDB resulted in poor sensitization. The reaction proceeded satisfactorily in a water bath at 24° C.

†Although these extracts are prepared according to a standard method, for optimal sensitization slightly different amounts were required with different lots of extracts of the same pollen. Prior to sensitization of red cells, the pollen extracts were diluted to a final volume of 3 ml. with cold physiologic saline.

Performance of the hemagglutination test: For hemagglutination experiments, rows of lipped, round-bottomed* 10 mm. by 75 mm. test tubes were placed in a masonite rack provided with holes and supported on a metal frame.† This arrangement allowed the tubes to hang vertically, which was essential for uniform sedimentation of the red cells in all tubes. The hemagglutination patterns could be observed easily when a mirror was placed below the tubes.

To remove nonspecific agglutinins, all sera were absorbed out with an equal volume of packed, nonsensitized rabbit erythrocytes (washed three times with cold saline) for one to two hours at room temperature or overnight in the cold. The cells used for absorption and sensitization were always from the same rabbit. The absorbed antisera were serially diluted in halving dilutions with diluent, each tube containing 0.5 ml. A volume of 0.05 ml. of sensitized cell suspension was added to each tube. The tubes were gently shaken to disperse the cells. The reaction proceeded at room temperature and could be read after a minimum of three hours. Usually they were read after twelve hours, although the patterns remained unchanged for at least thirty hours at room temperature or for several days in the cold.

The titer of the antiserum was expressed as the reciprocal of the highest dilution of antiserum which gave a positive pattern. The hemagglutination patterns were graded according to the recommendations of Stavitsky.[23] Because the end point of the hemagglutination is somewhat subjective, each reading was compared against a control row of sensitized cells in diluent and read by two observers. In addition, in each experiment the following controls were included: (1) To ascertain complete removal of nonspecific agglutinins, the sera were tested with nonsensitized rabbit cells‡ or with cells sensitized with heterologous protein antigens, for example, BSA and ovalbumin. (2) For day-to-day comparison of the method, a serum of known hemagglutination titer was employed.

Inhibition of hemagglutination: For inhibition tests, varying amounts of the appropriate antigen in 0.1 ml. glycerin-saline solution were added to each tube containing the antiserum in serial dilution. As a control, 0.1 ml. of saline and/or glycerin was added to an identical series of tubes. Sensitized red cells (0.05 ml.) were added to all tubes and the reaction was read as before.

RESULTS

Hemagglutination With Rabbit Antigrass Sera.—None of the antisera obtained after the first course of immunization contained precipitating antibodies in amounts detectable by ring test. However, all sera had hemagglutination titers ranging from 60 to 15,000, the majority being in the range of 500 to 2,000.

Following the second course of immunization, all rabbit antisera contained precipitating antibodies, as evidenced by positive ring tests. These antisera

*Round-bottomed tubes are preferred, since cells form irregular patterns in flat-bottomed tubes.

†Made of Dexion angle aluminum.

‡0.1 ml. of 50 per cent red cell suspension was made up in diluent to a final volume of 2.5 ml.

were tested with erythrocytes sensitized not only with the homologous antigens but also with the heterologous grass pollen extracts and with water-soluble extract of ragweed pollen (WSR).[24] The highest hemagglutination titers, ranging from 2,500 to 77,000 were obtained with the homologous systems, the majority of the sera giving titers of 10,000 to 40,000 (Table I). There were considerable cross-reactions among the five grasses—June grass, orchard grass, sweet vernal, timothy, and redtop. It is worth noting that occasionally the antiserum to any one of the grasses gave equal or even slightly higher titers when tested with cells sensitized with one of the other four grasses. On the other hand, with the antisera to the five grasses minimal reactions, if any, were obtained with cells sensitized with Bermuda grass or ragweed. Only slight reactions were obtained between antisera to ragweed and cells sensitized with the extracts of the six grass pollens. Similarly, antisera to Bermuda grass gave only slight reactions with the other six antigenic systems. The various cross-reactions are evident in Table I.

TABLE I. CORRELATION OF POLLEN ANTIGENS USING BDB-HEMAGGLUTINATION TECHNIQUE*

RABBIT ANTISERUM TO:	RABBIT NO.	HEMAGGLUTINATION TITERS OBTAINED WITH CELLS SENSITIZED WITH						
		JUNE	SWEET VERNAL	TIMOTHY	ORCHARD	REDTOP	BERMUDA	RAGWEED
June	1	38,400	9,600	38,400	19,200	38,400	600	300
	3	19,200	2,400	19,200	19,200	19,200	300	300
	17	4,800	300	1,200	9,600	1,200	0	0
Sweet vernal	4	9,600	19,200	38,400	38,400	19,200	1,200	600
	5	9,600	19,200	38,400	9,600	9,600	0	600
	6	9,600	19,200	19,200	9,600	9,600	0	1,200
Timothy	7	1,200	600	4,800	9,600	1,200	300	600
	8	600	600	2,400	4,800	1,200	300	300
	9	600	600	4,800	4,800	300	600	600
Orchard	11	2,400	2,400	9,600	38,400	2,400	600	1,200
	19	600	4,800	9,600	76,800	2,400	600	600
	20	600	1,200	2,400	19,200	600	1,200	600
Redtop	12	2,400	1,200	4,800	4,800	9,600	0	0
	13	300	300	1,200	2,400	9,600	300	600
	14	300	300	1,200	4,800	4,800	0	300
Bermuda	23	600	600	1,200	1,200	600	9,600	600
	24	300	300	2,400	600	600	38,400	300
	29	300	1,200	1,200	1,200	600	19,200	300
Ragweed	2	0	300	300	300	0	0	9,600
	21	300	300	1,200	300	0	1,200	9,600
	35	600	300	1,200	600	600	600	38,400

*The sera used for this correlation were obtained from rabbits after the second course of immunization. The titers of homologous antigen-antibody systems are given in the rectangles. Zero indicates that no hemagglutination was obtained with sera diluted 300-fold.

Specificity of Hemagglutination.—The hemagglutination was shown to be the result of the specific reaction between sensitized cells and homologous antibodies. This was demonstrated in two ways: (1) by the absence of agglutination of red blood cells sensitized with unrelated antigens (BSA, ovalbumin) and

(2) by inhibition experiments. With most antisera the addition of 8 to 10 μg of free antigen in 0.1 ml. of glycerin-saline solution completely inhibited the reactions. With two strong sera (sweet vernal No. 4 and June No. 1, Table I) that had hemagglutination titers of 20,000 and 40,000, complete inhibition was achieved with about 18 μg. In contrast, all antitimothy sera, in spite of their rather low hemagglutination titers required larger quantities of the timothy extract, that is, of the order of 19 μg, for complete inhibition. In control experiments in which saline or glycerin was used instead of the antigen, no inhibition was observed.

TABLE II. INHIBITION OF HEMAGGLUTINATION WITH RABBIT ANTISERA*

RABBIT ANTISERUM TO:	RABBIT NO.	TESTED WITH CELLS SENSITIZED WITH:	MICROGRAMS OF FREE ANTIGENS REQUIRED FOR COMPLETE INHIBITION†						
			JUNE	SWEET VERNAL	TIMOTHY	ORCHARD	REDTOP	BERMUDA	RAGWEED
June	1	June	1.7	53.3	56.7	15.6	[125]	[175]	[600]
	3		1.7	26.6	226.8	124.8	[125]		
	17		0.3	3.2	6.8	3.7	7.8		
Sweet vernal	4	Sweet vernal	2.8	0.1	3.0	3.2	1.7	[175]	[3,000]
	5		1.4	0.1	6.0	1.6	3.3		
	6		1.4	0.2	6.0	3.2	1.7		
Timothy	7	Timothy	2.8	[380]	3.0	12.8	207.0	[175]	[1,000]
	8		5.6	44.4	1.5	3.2	3.3		
	9		1.4	1.4	0.3	1.6	1.7		
Orchard	11	Orchard	5.6	11.1	6.0	0.2	13.2	[175]	[5,000]
	19		2.8	44.4	12.0	0.8	6.6		
	20		2.8	5.6	12.0	0.2	13.2		
Redtop	12	Redtop	5.6	22.2	24.0	208.0	0.2	[175]	[1,000]
	13		11.2	22.2	24.0	6.4	0.2		
	14		44.8	44.4	12.0	208.0	0.2		
Bermuda	23	Bermuda	[105]	[105]	[215]	[125]	[125]	1.7	[1,500]
	24							6.5	
	29							1.7	
Ragweed	2	Ragweed	[175]	[175]	[380]	[215]	[215]	[175]	8
	21								8
	35								4

*These sera were diluted 2,000-fold.

†No inhibition was obtained with the amounts of antigens given in brackets. In these experiments higher concentrations of antigens were not used.

To gain further information about the antigenic relationship among the different pollens, we resorted to an inhibition technique, using the antiserum in a constant dilution with decreasing amounts of antigen. Table II records the minimum quantities of the inhibitors needed for complete inhibition of hemagglutination with 1:2,000 diluted antisera. As can be seen, the hemagglutination was inhibited with minute amounts of the homologous antigens. For inhibition of the hemagglutination of each of the five homologous systems (June, orchard, timothy, sweet vernal, and redtop), however, somewhat higher concentrations of the other four grass antigens were required. Moreover, these reactions could not be inhibited with much higher amounts of Bermuda grass or ragweed pollen extracts. Similarly, the anti-Bermuda and antiragweed systems could not be inhibited with large doses of any of the other pollen extracts, even if these two antisera were further diluted to 1:10,000. From these results, it

appears that both Bermuda grass pollen and ragweed pollen contain distinctly different antigens, whereas the other five grasses appear to have common antigen(s) to a varying degree.

Hemagglutination With Human Grass-Sensitive Sera.—The hemagglutination titers of the thirty allergic sera investigated in the present study are presented in Table III. As can be seen, twelve untreated and sixteen treated sera gave hemagglutination titers ranging up to 384 and 1,536, respectively. Generally, higher titers were observed with treated sera. Most of the patients were sensitive also to ragweed, as demonstrated by positive hemagglutination and direct skin tests. In seven cases, however, no ragweed sensitivity could be demonstrated by either of the tests. Only two sera (one treated and one untreated) failed to show hemagglutination with any of the six grasses, though the patients gave positive skin tests.

TABLE III. HEMAGGLUTINATION TITERS OF HUMAN SERA FROM GRASS-SENSITIVE PATIENTS

PATIENT	HEMAGGLUTINATION TITERS OBTAINED WITH CELLS SENSITIZED WITH						
	JUNE	SWEET VERNAL	TIMOTHY	ORCHARD	REDTOP	BERMUDA	RAGWEED
				Untreated			
M. G.	96	12	192	192	96	24	192
R. L.	24	24	6	6	48	0	6
J. P.	96	48	96	96	96	48	192
N. P.	40	0	10	160	20	0	0
J. R.	40	0	20	20	20	0	160
R. S.	24	48	12	24	24	96	0
M. V.	384	24	192	96	384	24	192
F. W.	5	0	10	5	0	0	0
P. S.	40	0	20	20	20	0	0
P. G.	5	20	10	20	20	20	10
G. B.	160	20	320	40	20	20	80
G. A.	40	10	80	40	10	10	80
J. N.	0	0	0	0	0	0	0
				Treated			
A. B.	0	24	24	12	0	48	192
P. B.	6	0	24	48	48	48	192
M. B.	48	48	96	96	96	24	192
H. C.	96	96	48	384	384	96	12
C. H.	384	192	192	384	192	96	96
F. H.	768	192	768	192	1,536	48	96
L. H.	192	384	192	96	384	192	96
A. L.	96	48	192	96	96	0	0
C. M.	40	48	160	40	48	24	48
S. S.	48	24	24	12	24	24	24
C. T.	48	24	24	24	96	24	0
A. T.	96	96	96	24	96	48	0
K. T.	80	40	40	20	40	10	20
G. T.	96	48	48	48	48	96	192
M. M.	20	0	0	5	40	0	40
A. D.	40	0	10	20	10	0	40
H. D.	0	0	0	0	0	0	0

Zero indicates that no hemagglutination was obtained with the undiluted serum.

As part of the test for the specificity of the hemagglutination technique with regard to human antigrass sera, thirty-seven sera from normal subjects (who gave negative intradermal skin tests to a mixture of the six grass pollen extracts) were tested by hemagglutination. Only one of these sera gave

hemagglutination. The titers obtained with this serum were 4, 8, and 16 with June grass, sweet vernal, and timothy, respectively. However, none of the other thirty-six sera gave any reaction with any of the six grasses. The specificity of this reaction was further demonstrated, as with rabbit antisera, by inhibiting the hemagglutination specifically with free soluble grass antigen(s). When 8 to 10 μg of inhibitor was used, the hemagglutination was completely inhibited in about one-half of the allergic sera, regardless of the titer. In the other sera the titers were markedly reduced. When larger amounts of inhibitor (17 to 40 μg) were used, all the reactions were completely inhibited. These results are shown in Table IV.

TABLE IV. INHIBITION OF HEMAGGLUTINATION WITH HUMAN ALLERGIC SERA

	HEMAGGLUTINATION TITERS AFTER INHIBITION WITH													
	JUNE		SWEET VERNAL		TIMOTHY		ORCHARD		REDTOP		BERMUDA		RAGWEED	
PATIENT	9 μg	36 μg	9 μg	36 μg	19 μg	74 μg	10 μg	40 μg	10 μg	40 μg	9 μg	36 μg	50 μg	100 μg
	Untreated													
M. G.	0		0		0		0		0		0		0	
R. L.	0		0		0		0		0		0		0	
J. P.	12	0	6	0	6	0	48	0	12	0	6	0	0	
N. P.	0		0		0		0		0		0		0	
J. R.	0		0		0		0		0		0		0	
R. S.	6	0	6	0	6	0	0		6	0	12	0	0	
M. V.	0		0		0		0		0		0		0	
P. S.	0		0		0		0		0		0		0	
P. G.	0		0		0		0		0		0		0	
	Treated													
A. B.	0		24	0	12	0	0		0		0		0	
P. B.	0		0		0		12	0	12	0	0		0	
M. B.	0		12	-	0		24	-	6	-	12	-	0	
H. C.	24	0	24	0	12	0	24	0	12	0	24	0	0	
C. H.	0		24	0	12	0	12	0	0		48	0	0	
F. H.	96	0	48	0	48	0	96	0	96	0	48	0	0	
L. H.	96	0	48	0	48	0	6	0	96	0	48	0	24	0
A. L.	0		12	0	0		0		0		0		0	
C. M.	6	0	12	0	6	0	12	0	6	0	12	0	12	0
S. S.	24	0	12	0	12	0	6	0	12	0	12	0	0	
C. T.	6	0	6	0	0		12	0	12	0	12	0	0	
A. T.	24	-	12	-	0		0		6	-	0		0	
K. T.	24	0	12	0	0		0		6	0	0		0	
G. T.	24	-	24	-	12	-	12	-	12	-	12	-	6	-
M. M.	0		0		0		0		0		0		0	

Zero indicates complete inhibition of hemagglutination.

Correlation of the Allergens of Different Grasses.—In a previous study from this laboratory[25] it was reported that skin-sensitizing and/or blocking antibodies could be removed completely from the sera of ragweed-sensitive subjects by absorption with antigenically specific adsorbents prepared by coupling the constituents of a water-soluble extract of ragweed pollen to polystyrene or to erythrocytes via stable covalent azo bonds. The removal of these antibodies was always accompanied by a parallel disappearance of the hemagglutinating factor.

In the present study the same methods have been applied to investigate the relationship between the hemagglutinating factor(s) and the skin-sensitizing and/or blocking antibodies in the sera of grass-sensitive subjects. Furthermore,

using adsorbents prepared with individual grass pollen extracts, the relationship among the allergens of the six pollens used could also be studied. The experimental procedures were similar to those employed in the ragweed system with sensitized erythrocytes[21] and polystyrene-antigen conjugates.[26] For these experiments, water-soluble extracts of each of the six defatted grass pollens were prepared.

It was shown that any of the five grasses (June, sweet vernal, timothy, orchard, and redtop) coupled to erythrocytes or to polystyrene removed the antibodies from the serum of a treated grass-sensitive subject. This is illustrated by the experiments recorded in Table V. Total removal of antibodies was achieved with adsorbents to which large amounts of the allergenic constituents were coupled (columns 1, 4, and 6 in Table V). On certain occasions, when an insufficient amount of adsorbent was used, the serum was only partially depleted of antibodies (compare columns 1 and 2 in Table V). In these cases the hemagglutination titers of the absorbed sera decreased with respect not only to the grass pollen which had been coupled to the inert supporting media but also to the other four grasses (column 7 in Table V). From these findings, one can conclude that the pollen extracts of the five grasses contain common allergenic constituents. In contrast, Bermuda grass appeared to be allergenically distinct from the other five grasses, inasmuch as no hemagglutination was obtained with red cells sensitized with the pollen extract of Bermuda grass and the serum of a patient (A. L.) who was allergic to the other five grasses. Moreover, adsorbents prepared with Bermuda grass failed to remove the hemagglutination factors from sera of patients sensitive to grasses other than Bermuda. Similarly, with the serum containing no antibodies to ragweed (Patient A. L.), the specificity of the absorption method was tested. Column 8 in Table V shows that incubation of this serum with a polystyrene-ragweed conjugate failed to remove antibodies to grass pollens. Similar findings were also reported by Gordon and associates[21] who coupled ragweed allergens to erythrocytes.

TABLE V

TESTED WITH CELLS SENSITIZED WITH	HEMAGGLUTINATION TITERS OF AN ALLERGIC SERUM								
	BEFORE ABSORPTION	AFTER ABSORPTION WITH							
		JUNE		SWEET VERNAL	TIMOTHY	ORCHARD	REDTOP		RAGWEED
		1*	2†	3†	4†	5‡	6†	7§	8†
June	160	0	10	40	0	0	0	40	160
Sweet vernal	20	0	0	5	0	0	0	10	20
Timothy	80	0	0	20	0	20	0	40	40
Orchard	160	0	5	10	0	0	0	80	160
Redtop	40	0	5	20	0	0	0	10	20
Bermuda	0	0	0	0	0	0	0	0	0

Zero indicates no hemagglutination.
*Water-soluble grass constituents coupled to erythrocytes.
†Water-soluble grass constituents coupled to polystyrene.
‡Glycerin-saline-soluble grass constituents coupled to erythrocytes.
§Glycerin-saline-soluble grass constituents coupled to polystyrene.

In another experiment, the treated serum (Patient A. L.) was absorbed with erythrocytes sensitized with the dialyzed residue of a water-soluble June

extract. All antibodies to this residue were removed, as evidenced by the negative hemagglutination test obtained with cells sensitized with it. However, this apparently completely absorbed serum showed the presence of considerable amounts of antibodies when tested with cells sensitized with the whole water-soluble extract of June grass pollen; similar results were obtained with cells sensitized with the glycerin-saline extracts of the other grass pollens. This finding would suggest that different antibodies produced to different allergenic constituents of one and the same pollen may be demonstrated with the hemagglutination technique.

DISCUSSION

This study indicates clearly the applicability of the BDB-hemagglutination technique for the demonstration of antibodies to grass pollen constituents both in sera of immunized rabbits and in sera of grass-sensitive patients.

This method is highly sensitive. Thus, in the present study rabbit antisera containing precipitating antibodies which could not be detected in dilutions higher than 1:50 gave hemagglutination titers of the order of 10^4.* Furthermore, with weaker rabbit antisera, particularly after the first course of immunization, which failed to give any ring test, hemagglutination titers of the order of 10^2 to 10^3 were still obtained. Although most allergic sera displayed hemagglutination titers of the order of 10^2, the incidence of high titers was greater in the group of treated sera (Table III). Similar findings have already been reported from this laboratory[21] for sera of ragweed-sensitive patients.

The absorption of nonspecific agglutinins with nonsensitized cells resulted in hemolysis, particularly excessive with fresh sera. This made the precise evaluation of the hemagglutination titer impossible, especially with weak sera. This difficulty was overcome by pretreating the serum with precipitating antibody-antigen complexes or zymosan or by heating it. The hemagglutination titer was not affected by any of these procedures which presumably lead to inactivation of complement. With the same batch of sensitized red cells, identical titers were obtained in a series of experiments using the same serum. With different preparations of sensitized cells, the titers were reproducible within ± 2 tubes (equivalent to a factor of 16). Therefore, one cannot attach too much significance to the absolute value of the hemagglutination titer, particularly if one has not exhaustively determined the optimal conditions for sensitization of red cells. It is to be emphasized that the end point in the titration of the hemagglutinating factor(s) must be established in terms of the patterns obtained in the control series of tubes containing normal serum. Nevertheless, the relationship among the titers of different sera is maintained from experiment to experiment, provided that the tests are done concurrently with all the sera and with the same batch of sensitized cells.

The specificity of the method was demonstrated by inhibition experiments. The results listed in Table II indicate that hemagglutination given by ragweed-sensitized cells and antiragweed sera was inhibited only by free soluble ragweed

*In previous studies [18, 21] with stronger rabbit antisera, hemagglutination titers of the order of 10^6 to 10^7 were also obtained.

extract. Similarly, the hemagglutination obtained with the Bermuda-anti-Bermuda system was inhibited only by free Bermuda antigens. However, the reactions obtained with cells sensitized with each of the five grasses—June grass, sweet vernal, timothy, orchard grass and redtop—and the corresponding five antisera could be inhibited with any one of these five grass pollen extracts but not with Bermuda grass or ragweed antigens. These results are interpreted as indicating that the group of five grasses contains common antigens which are distinct from those of Bermuda grass and ragweed.

The cross-reactions demonstrated for the group of the five grasses were obtained with antisera diluted 1:2,000. This dilution was arbitrarily chosen in view of the limited amounts of antisera and antigens which were available at the time of these experiments. It should be pointed out that the conclusions reached with diluted sera may be fallacious inasmuch as the concentration of some of the cross-reacting antibodies may be inadvertently lowered to a level below that required for their detection. The weakness of this method for establishing antigenic cross-reactions is borne out by comparing the results of Table II with those of Table I. Thus, from the results of the inhibition studies with 2,000-fold-diluted sera (Table II), it would appear that Bermuda grass and ragweed contain antigens different from those of the other five grasses. In contrast, it is apparent from Table I that Bermuda grass and ragweed contain a very small, but detectable, amount of antigens common to the other five grasses.

TABLE VI. INHIBITION OF HEMAGGLUTINATION WITH A RABBIT ANTIRAGWEED SERUM*

CELLS SENSITIZED WITH	MICROGRAMS OF FREE ANTIGENS REQUIRED FOR COMPLETE INHIBITION:						
	JUNE	SWEET VERNAL	TIMOTHY	ORCHARD	REDTOP	BERMUDA	RAGWEED
June	9.0	9.0	4.5	9.0	4.5	38.0	38.0
Sweet vernal	9.0	9.0	9.0	4.5	19.0	19.0	19.0
Timothy	4.5	38.0	2.2	4.5	2.2	9.0	19.0
Orchard	2.2	4.5	4.5	9.0	9.0	9.0	75.0
Redtop	4.5	4.5	2.2	4.5	4.5	19.0	19.0
Bermuda	△	△	△	△	△	4.5	75.0
Ragweed	△	△	△	△	△	△	19.0

*This serum was diluted 100-fold.
△ = No inhibition was obtained with 600 μg of free antigen.

Unfortunately, because of lack of materials, the possible cross-reactions among all these different systems could not be investigated with undiluted sera by the inhibition technique. Only one such experiment was done with a 100-fold-diluted rabbit antiragweed serum.* Hemagglutination was obtained with this serum and cells sensitized with each of the six grasses, and it was completely inhibited with soluble ragweed extract. (See Table VI.) The hemagglutination given by cells sensitized with the five grasses (June grass, sweet vernal, timothy, orchard grass, and redtop) and this serum could be inhibited by the pollen constituents of any of these five grasses, as well as by Bermuda grass and ragweed antigens. On the other hand, the hemagglutination resulting with

*In this experiment serum No. 35 of Table I was used. As seen from this table, cross-reactions could still be detected between this serum (even in dilutions higher than 1:300) and the other heterologous antigens.

cells sensitized with Bermuda antigens could be inhibited only by the antigens of Bermuda grass or ragweed pollens. Moreover, the hemagglutination with cells sensitized with ragweed could be inhibited only with ragweed pollen extracts. These results therefore would suggest that (1) the five grasses (June grass, sweet vernal, timothy, orchard grass, and redtop) contain only small amounts of some of the antigens of ragweed pollen; (2) while Bermuda grass contains a small amount of antigens common to the other grasses, the majority of its constituents are antigenically distinct; (3) Bermuda grass contains a small amount of constituents antigenically similar to those found in ragweed; (4) ragweed contains only a small amount of constituents antigenically similar to those found in the six grasses, in addition to its distinct antigens. These results, although not based on an exhaustive study, demonstrate the usefulness of the hemagglutination technique in establishing antigenic relationships among related systems.

As with rabbit antisera, the specificity of the hemagglutination method for detection of antibody-like factor(s) in sera of grass-sensitive persons was demonstrated by inhibition of the reaction with free soluble grass pollen extracts. For complete inhibition of hemagglutination, however, larger quantities of the inhibitors were required than the quantities needed for inhibition of hemagglutination obtained with rabbit antisera containing precipitating antibodies. This would, therefore, suggest that the allergenic constituents of grass pollens are present in smaller concentrations than those responsible for antigenicity in rabbits and that there is no justification to assume that antigenically determinant groups are also responsible for inducing the production of antibodies in allergic persons. Unfortunately, in several previous studies no discrimination was made between antigenicity and allergenicity of grass pollens and it was implied that the allergenic components were identical to the grass pollen constituents which were antigenic in rabbits.[27, 28]

Regarding the specificity of the method, it is important to note that among the thirty-seven normal human sera used only one gave a low titer of about 16 with grass-sensitized cells. This person had been previously used as a subject for Prausnitz-Küstner tests and might have developed antibodies subsequent to injection with pollen extracts. Similarly, only two of these thirty-seven sera gave hemagglutination with ragweed-sensitized cells. One of these two sera belonged to the same person used as a Prausnitz-Küstner subject. On the other hand, out of thirty sera from grass-sensitive persons (Table III) only two failed to give hemagglutination with any of the six grasses, although their donors gave positive skin tests.*

The specificity of the method was further demonstrated by removal of the hemagglutinating factor(s) from one allergic serum by absorption with allergenic adsorbents. As shown in Table V, the hemagglutinating factor(s) were removed by adsorbents prepared by coupling grass pollen constituents to polystyrene or erythrocytes. It was also found by Prausnitz-Küstner tests that the skin-sensitizing antibodies of this serum were removed by this treatment

*Unfortunately, these two sera were not tested for the presence of skin-sensitizing antibodies by Prausnitz-Küstner tests. It is possible that the concentration of circulating antibodies in these sera was below the amount detectable by the hemagglutination technique.

and that removal of the hemagglutinating factor with respect to any of the allergens was paralleled by a partial or complete reduction in the Prausnitz-Küstner titers. These results, therefore, demonstrate that skin-sensitizing as well as hemagglutinating factor(s) combine in vitro with the grass pollen constituents and that these grasses may possess different amounts of identical or unrelated allergens.

Similar observations were previously made[21] with sera of ragweed-sensitive persons, and it was then concluded that the hemagglutinating factor(s) may be identified with skin-sensitizing and/or blocking antibodies present in untreated and treated sera, respectively. At stated in earlier publications,[25, 30] however, it should be stressed that as yet there is no unequivocal proof that skin-sensitizing and hemagglutinating properties of untreated sera are different attributes of one and the same serum molecule; they may well be associated with distinct serum components. Similar conclusions were drawn from the relationship between hemagglutinating and blocking capacities of treated sera. In view of the limited number of Prausnitz-Küstner tests done in this study, it is impossible to draw any more definitive conclusion regarding the nature of the hemagglutinating factor(s) and its relationship to skin-sensitizing antibodies of sera from grass-sensitive patients.

APPENDIX

In view of the danger of transferring homologous serum hepatitis by Prausnitz-Küstner tests, only one serum was used in this study for establishing the allergenic relationship among the different grasses. This serum (A. L.) was obtained from a physician who has never had any symptoms suggestive of hepatitis. The results of cross-neutralization experiments using desensitizing doses of each of the five grasses (June grass, timothy, orchard grass, redtop, and Bermuda grass) with this serum are given in Table VII. Cross-neutralization with sweet vernal was not attempted, since at the time these experiments were performed our supply of sweet vernal had been exhausted. The desensitizing dose with respect to each allergen was established as described previously for ragweed allergens.[29]

From these results, one may draw the following conclusions: (1) redtop grass pollen appears to possess all the allergens common to the other grasses; (2) this serum contained a small amount of antibodies directed specifically against Bermuda grass allergens; (3) all the other grasses contain additional allergens to those found in Bermuda grass pollen; and (4) June grass, timothy, and orchard grass have a complex and in many respects a similar allergenic composition. Since these conclusions are based on the pattern of reactivity given by one allergic serum, they may not be valid for other allergic sera from

TABLE VII

SITE DESENSITIZED WITH	SKIN REACTIONS AFTER CHALLENGE WITH				
	REDTOP	JUNE	TIMOTHY	ORCHARD	BERMUDA
Redtop	0	0	0	0	0
Bermuda	+	+	+	+	0
June	+	0	0	0	+
Timothy	+	+	0	0	+
Orchard	0	0	+	0	+

grass-sensitive persons. Nevertheless, these experiments clearly demonstrate the allergenic complexity of these grass pollens and the necessity of using precisely defined allergenic fractions in cross-neutralization studies.

SUMMARY

The BDB-hemagglutination technique has been adapted for the demonstration of antibodies to grass pollen constituents in the sera of immunized rabbits and grass-sensitive hay fever patients. The grasses used were timothy, June grass, orchard grass, redtop, sweet vernal, and Bermuda grass. The method was shown to be highly sensitive. Titers of the order of 10^4 and 10^2 were obtained with sera of immunized rabbits and of allergic human subjects, respectively. The specificity of the technique was demonstrated by inhibition of the hemagglutination with free soluble pollen constituents.

The inhibition of hemagglutination obtained with rabbit antisera was used for establishing antigenic relationships among the various grasses. Timothy, June grass, orchard grass, redtop, and sweet vernal were found to contain common antigens, which were different from those of Bermuda grass and ragweed. The results of Prausnitz-Küstner tests using the cross-neutralization technique with desensitizing doses of each of the five grasses (timothy, June grass, orchard grass, redtop, and Bermuda grass) suggest that redtop grass pollen contains all the allergens common to the other grasses and that timothy, June grass, and orchard grass pollens have a complex and in many respects similar allergenic composition and contain additional allergens to those found in Bermuda grass pollen.

Absorption of an allergic serum with adsorbents prepared by coupling grass pollen constituents to polystyrene or erythrocytes resulted in the removal of the hemagglutinating factor(s), which was paralleled by the removal of skin-sensitizing antibodies.

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REFERENCES

1. Prausnitz, C., and Küstner, H.: Studien über die Ueberempfindlichkeit, Centralbl. f. Bakt. **86**: 160, 1921.
2. Grabar, P.: Comparaison entre les methodes immunologiques quantitatives et bilan de leurs applications (Abst.), 6th International Congress of Microbiology, Rome, vol. 1, p. 477, 1953.
3. Miller, H., and Campbell, D. H.: Reagins: Preliminary Report on Experimental Evidence in Support of a New Theory of Their Nature, Ann. Allergy **5**: 236, 1947.
4. Bukantz, S. C., Johnson, M. C., and Hampton, S.: Quantitative Immunologic Studies With Allergens. I. Colorimetric Absolute Methods in the Analysis of Factors Influencing Precipitation of Ragweed Pollen Extracts and Homologous Rabbit Antisera, J. ALLERGY **20**: 1, 1949.
5. Orlans, E. S., Rubinstein, L. J., and Marrack, J. R.: Detection of Antibodies in the Serum of Patients Suffering From Hay Fever, Acta allergol. **6**: 33, 1953.
6. Cavelti, P. A.: Complement Fixation Studies in Allergy, J. ALLERGY **21**: 532, 1950.
7. Hensel, M. E., and Sheldon, J. M.: Complement Fixation With Specific Allergens in Hay Fever. I. Problem, Procedure, Diagnostic Value, J. Lab. & Clin. Med. **26**: 1586, 1940-1941.
8. Albus, G.: Beeinflussung der Antikörperkonzentration durch spezifische Desensibilizierung bei Pollenallergie, Ztschr. f. d. ges. exper. Med. **95**: 703, 1935.

9. Portnoy, J., and Sherman, W. B.: Complement Fixation Studies in Ragweed Allergy. II. Determination of Antibody in Human Sera to Ragweed Antigen by Means of a Complement Fixation Inhibition Test; the Relationship of Antibody so Determined to the Passive Transfer for Blocking Antibody, J. ALLERGY **25**: 229, 1954.
10. Loeb, L. F.: Über die chemische Natur der Allergene. III. Pollen von Alopecurus (Fuchsschwanzgras), Klin. Wchnschr. **8**: 926, 1929.
11. Hampton, S. F., Stull, A., and Cooke, R. A.: Antigenic Studies by the Dale Test. I. The Antigenic Relationship of Certain Pollens, J. ALLERGY **10**: 417, 1938-1939.
12. Ovary, Z., and Biozzi, G.: Passive Sensitization of Skin of Guinea Pig With Human Antibody, Internat. Arch. Allergy **5**: 241, 1954.
13. Jones, F. S.: Agglutination by Precipitin, J. Exper. Med. **48**: 183, 1928.
14. Boyden, S. V.: The Adsorption of Proteins on Erythrocytes Treated With Tannic Acid and Subsequent Hemagglutination by Antiprotein Sera, J. Exper. Med. **93**: 107, 1951.
15. Kallós, P., and Kallós-Deffner, L.: Specific Serum-Agglutination of Corpuscles (Collodion-Pellets or Erythrocytes) Coated With Allergenic Material, Internat. Arch. Allergy **2**: 70, 1951.
16. Feinberg, R. J., Davison, J. D., and Flick, J. A.: The Detection of Antibodies in Hay Fever Sera by Means of Hemagglutination, J. Immunol. **77**: 279, 1956.
17. Feinberg, R. J., and Flick, J. A.: Elution of Pollen Antigens From Tannic Acid Treated Erythrocytes, Proc. Soc. Exper. Biol. & Med. **96**: 71, 1957.
18. Sehon, A. H., Gordon, J., and Rose, B.: Antibodylike Factor in Serums of Ragweed-Sensitive Individuals Shown In Vitro, Science **125**: 597, 1957.
19. Pressman, D., Campbell, D. H., and Pauling, L.: The Agglutination of Intact Azoerythrocytes by Antisera Homologous to the Attached Groups, J. Immunol. **44**: 101, 1942.
20. Stavitsky, A. B., and Arquilla, E. R.: Micromethods for the Study of Proteins and Antibodies. III. Procedure and Applications of Hemagglutination and Hemagglutination-Inhibition Reactions With Bis-diazotized Benzidine and Protein-conjugated Red Blood Cells, J. Immunol. **74**: 306, 1955.
21. Gordon, J., Rose, B., and Sehon, A. H.: Detection of "Non-Precipitating" Antibodies in Sera of Individuals Allergic to Ragweed Pollen by an In Vitro Method, J. Exper. Med. **108**: 37, 1958.
22. Wall, R. L., Jayne, M., and Beauchamp, J.: Preservation of the Specific Agglutinability of Human Red Blood Cells, J. Lab. & Clin. Med. **36**: 378, 1950.
23. Stavitsky, A. B.: Micromethods for the Study of Proteins and Antibodies. I. Procedure and General Applications of Hemagglutination and Hemagglutination-Inhibition Reactions With Tannic Acid and Protein-Treated Red Blood Cells, J. Immunol. **72**: 360, 1954.
24. Sehon, A. H., Richter, M., Harter, J. G., and Rose, B.: Studies on Ragweed Pollen. I. Preliminary Fractionation of Ragweed Pollen; Characterization and Allergenicity of the Isolated Fractions, J. ALLERGY **27**: 236, 1956.
25. Sehon, A. H.: Different Types of Antibodies Produced by Allergic Individuals Depending on Route of Immunization (to be published in extenso in June, 1960, in the Proc. Internat. Symposium on "Mechanism of Antibody Formation" held in Prague, May, 1959).
26. Gyenes, L., Rose, B., and Sehon, A. H.: Isolation of Antibodies on Antigen-Polystyrene Conjugates, Nature, London **181**: 1465, 1958.
27. Wodehouse, R. P.: Antigenic Analysis by Gel Diffusion. 2. Grass Pollen, Internat. Arch. Allergy **6**: 65, 1955.
28. Gosselin, M. L., Mynors, L. S., and Coombs, R. R. A.: Serological Studies on Pollen Extracts. II. Demonstration by a Serological Method of the Specificity of the Antigen-Mosaic of the Extracts of Six Grass Pollens, Acta allergol. **6**: 96, 1953.
29. Richter, M., Harter, J. G., Sehon, A. H., and Rose, B.: Studies on Ragweed Pollen. III. Estimation of the Minimum Number of Allergens in the Water-Soluble Extract of Ragweed Pollen and a Critical Evaluation of the Neutralization Technique; The Demonstration of Two Different Reagins in Sera of Ragweed-Sensitive Individuals, J. Immunol. **79**: 13, 1957.
30. Sehon, A. H.: Biophysical and Immunochemical Problems in Allergy, Proceedings of the Third International Congress of Allergology, Paris, 1958, Editions Flammarion, p. 209.

HOMOCHLORCYCLIZINE: AN ANTIALLERGIC DRUG WITH MULTIPLE CLINICAL PROPERTIES

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To counteract the known mechanisms responsible for the production of allergic manifestations, various drugs have been employed for temporary relief. These include vasoconstrictors, smooth muscle relaxers, antihistamines, anticholinergics, anti-inflammatory drugs, expectorants, and sedatives. There is reason to suspect that other mediators may also be involved in the mechanism of allergic symptoms. Among those suggested have been serotonin and slow-reacting substance (SRS). In anaphylaxis in the mouse and rat, serotonin has been shown to play a prominent role. In other species of animals there is less support for its place in anaphylaxis, and in man evidence for serotonin as a mechanism is virtually lacking.[1]

In screening a large number of chemical compounds which are modified structurally from Di-Paralene, Kimura, Young, and Richards[2] have selected one, N-p-chloro-benzhydryl-N′-methyl-homopiperazine dihydrochloride (homochlorcyclizine, or SA-97), that has a spectrum of pharmacologic actions expected to offer possible clinical usefulness. These properties include a high degree of antiserotonin activity, potent antihistaminic action, bronchodilator effect, mild anti-acetylcholine action, and antagonism to slow-reacting substance. In addition, the drug was shown to possess antifibrillatory and coronary dilator qualities and a certain degree of central stimulation.

METHOD OF CLINICAL STUDY

The drug* was administered to patients with various allergic manifestations, including asthma, allergic cough, perennial vasomotor rhinitis, seasonal hay fever, urticaria and angioedema, dermatitis, and dermographism. The general plan was to employ it in those patients who had failed to respond adequately to the usual drugs, other than anti-inflammatory agents. In some instances this compound was used as the first drug.

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Observations made comprised the general subjective and objective therapeutic effects of the drug, the speed of onset, the duration of action, dosage considerations, the side effects, the influence on local reactions from the injection of antigens for desensitization, and the effect on skin tests.

RESULTS

The therapeutic results are listed in Table I. A few words of explanation are pertinent to the grading. An "excellent" label means that SA-97 either completely relieved the condition or perfected the results as an addition to other therapy. The label "good" means that the drug was effective but that results were not complete or that its effectiveness was not better than with other drugs. "Fair" means that it is only moderately effective. The designation "poor" obviously implies that the drug is not useful.

TABLE I. THERAPEUTIC EFFECTS OF SA-97 ON ALLERGIC MANIFESTATIONS IN 346 PATIENTS

	TOTAL	EXCELLENT	GOOD	FAIR	POOR
Asthma	149	65	46	11	27
Cough	27	10	10	2	5
Perennial rhinitis	92	36	22	18	16
Seasonal hay fever	112	35	27	15	31
Urticaria and angioedema	24	10	10	1	3
Dermatitis	21	5	10	2	4
Dermographism	3	3	0	0	0
Pruritus	1	1	0	0	0
Gastrointestinal allergy	2	2	0	0	0
Sinus headache	4	2	1	1	0
Sunburn with edema	1	0	1	0	0
Migraine	3	0	0	0	3
Solar urticaria	1	0	0	0	1
Histamine cephalalgia	2	0	1	0	1

In the asthma group, it will be noted that about 75 per cent obtained satisfactory results. It should be emphasized here that in the vast majority of cases these were not acute asthma attacks. In the latter situations the drug is not very effective, at least in the manner used. The asthma patients mainly responsive to SA-97 were those who had persistent cough and wheezing of a moderate degree. The drug was particularly effective for the night symptoms and more dependable in children than in adults. The effect on allergic cough was especially noteworthy, although in three or four of those patients obtaining relief no allergy could be established. It is our impression that in the presence of respiratory infection the drug is not effective.

The patients with perennial allergic rhinitis were mainly those not responding to antihistamine therapy. Usually, protracted nasal blocking was a major indication for the use of this drug. About two-thirds of such patients obtained satisfactory results. Most of the seasonal hay fever patients had the ragweed type of hay fever, and the 55 per cent who responded well were mainly those failing to respond to antihistamines.

The urticaria and angioedema group consisted mainly of patients with chronic persistent types of the disease, most of whom did not respond to antihistamines and some of whom failed to respond to steroids. There were three

patients with acute symptoms. Of twenty-four patients, four gave inadequate responses. In half of the rest of the patients the lesions were completely subdued, and in the remainder the swellings were greatly diminished. In all but one, relief of the itching was obtained. Some of the urticaria patients were also treated with hydroxyzine. Some responded better to SA-97 and some to hydroxyzine. The antiedema effect was also evident in reduction of local swellings from desensitizing injections and in the diminution of skin test reactions. In a small group of patients with dermatitis, which included atopic, contact, and unknown types, there was effective relief of itching in the majority and diminution of the inflammation in some. Dermographism was completely controlled in a small group. There is a suggestion that the drug might be effective in pruritus without skin lesions and in some other allergic manifestations, such as those in the gastrointestinal tract. Of two patients with histamine cephalalgia, one obtained considerable benefit.

The dose used at first was 50 mg., as a rule given twice daily. After it became obvious that this schedule produced a high incidence of side effects, particularly in adults, the plan was revised. Most patients subsequently received 25 mg. twice daily, while in a few the morning dose was omitted, in others the bedtime dose was increased to 50 mg., and in a few 12.5 mg. doses were used. Because of the unpleasant taste and local irritation of the drug it was found best to have it specially coated. It is best not to take the drug on a completely empty stomach. Onset of action was fairly rapid, usually beginning in about a half-hour. The duration in most cases was between ten and sixteen hours.

TABLE II. SIDE EFFECTS OF SA-97 IN 346 PATIENTS

Total number of patients having side effects		78
Sedation, mild	31	
Sedation, moderate to severe	25	
Gastrointestinal, mainly nausea	18	
Weakness, fatigue	4	
Appetite depression	4	
Nervousness and tremor	3	
Dizziness	3	
Stimulation	6	
Disorientation	1	
Disturbed focus of vision	2	
Muscle cramps	1	
Dryness	3	

Table II shows the types and numbers of side effects encountered. While the figures indicate that about 22.5 per cent of the patients had side effects, it should be noted that this includes also those who in the first stages of the therapeutic trials took larger doses of the drug (see Table III). Of those receiving 25 mg. doses, 20 per cent experienced side effects. The most common symptom was sedation. In most cases this was either mild or it was overcome after several days and did not interfere with therapy. In some of the more severe cases the addition of methamphetamine hydrochloride or methylphenidate hydrochloride was sufficient to counteract the sedative effects. In a small group the sedative or other effects resulted in a discontinuance of the therapy. Three or four children showed irritability after prolonged use of the drug; this was quickly reversible after the drug's discontinuance.

The next most frequent side effects were gastrointestinal, mainly nausea. This was diminished after the tablets were coated. The remainder of the side effects consisted of a variety of reactions, with a few in each group. It is difficult to classify them, but some were apparently of central origin. As will be seen from Table III, side effects were more frequently noted in adults, even when the doses were the same as those which the children received.

TABLE III. SIDE EFFECTS FROM SA-97 RELATED TO DOSE AND AGE

DOSE (MG.)	NO SIDE EFFECTS		SIDE EFFECTS	
	CHILDREN	ADULTS	CHILDREN	ADULTS
50	20	36	4	30
25	86	121	15	35
12.5	6	---	2	1

DISCUSSION

It is evident that this drug is a valuable adjunct in the symptomatic treatment of a number of allergic manifestations. While it replaces neither antihistamines, bronchodilators, sympathomimetics, nor anti-inflammatory drugs, it has some actions not obtainable with any of the above.

The question arises: "What action of this drug is responsible for the symptomatic effects?" It is not its antihistaminic properties, since more potent antihistamines failed to relieve the edema of urticaria or nasal stuffiness of many of these cases. It cannot be purely bronchodilator action, since it was effective in some types of asthma or cough where more potent bronchodilators alone (epinephrine, isoproterenol, ephedrine, or aminophylline) did not suffice, and since in acute asthma or in the presence of infection bronchodilators were more effective. The moderate anti-acetylcholine action of this drug is not sufficient to explain its effectiveness. In a previous report we showed that hydroxyzine was very effective in urticaria,[3] yet that drug was of limited value in nasal and bronchial allergy. Hence, the mechanism of these two drugs (hydroxyzine and SA-97) must differ in some respect. Its sedative action cannot readily explain its effectiveness, since other more potent sedatives usually fail to relieve allergies. Serotonin has not been strongly implicated in human allergy,[1] nor have other compounds with high antiserotonin activity (hydroxyzine, Pyribenzamine,[3] Trilafon, Promazine, Phenergan, Benadryl, and Di-Paralene[4]) been effective in the range of manifestations relieved by this drug. The slow-reacting substance (SRS) has been shown to be released from the lung in anaphylaxis of man and guinea pig and has been demonstrated to produce contraction of human bronchial preparations in vitro.[5] Since Brocklehurst[6] has shown that this compound possesses an anti-slow-reacting action it is possible that this may explain its clinical effectiveness. Other possibilities must be considered, such as antagonism to bradykinin, Miles' permeability factor, or substance P, or the drug's effect on subcortical areas of the brain. An extended study of the pharmacologic and antiallergic properties of this and other new drugs may lead to better understanding of the mechanism of allergy.

SUMMARY

1. N-p-chloro-benzhydryl-N′-methyl-homopiperazine dihydrochloride is a drug possessing a wide range of clinical effect and is useful in certain types of asthma and allergic cough and resistant cases of seasonal and perennial allergic rhinitis, urticaria, dermatitis, and some other allergic and allergic-like manifestations.

2. Its clinical effectiveness cannot be satisfactorily explained by its known antagonism to histamine, acetylcholine, or serotonin or by simple bronchodilator action. It is possible that its action concerns antagonism against the slow-reacting substance or other known or unknown muscle-contracting or capillary permeability factors.

REFERENCES

1. Udenfriend, S., and Waalkes, T. R.: On the Role of Serotonin in Anaphylaxis, in Ford Symposium on Mechanisms of Hypersensitivity, Boston, 1959, Little, Brown & Co., p. 219.
2. Kimura, E. T., Young, P., and Richards, R. K.: Pharmacologic Properties of N-p-chlorobenzhydryl-N'-methyl-homopiperazine Dihydrochloride (Homochlorcyclizine; SA-97), a Serotonin Antagonist, J. ALLERGY **31**: 237, 1960.
3. Feinberg, A. R., Pruzansky, J. J., Feinberg, S. M., and Fisherman, E. W.: Hydroxyzine (Atarax) in Chronic Urticaria and in Allergic Manifestations, J. ALLERGY **29**: 358, 1958.
4. Kimura, E. T.: Personal communication.
5. Brocklehurst, W. E.: A Slow-Reacting Substance in Anaphylaxis—"SRS-A." In Ciba Foundation Symposium on Histamine, London, 1956, J. & A. Churchill, Ltd., p. 175.
6. Brocklehurst, W. E.: Personal communication.

PHARMACOLOGIC PROPERTIES OF N-p-CHLORO-BENZHYDRYL-N′-METHYL HOMOPIPERAZINE DIHYDROCHLORIDE (HOMOCHLORCYCLIZINE; SA-97), A SEROTONIN ANTAGONIST

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LARGE numbers of competitive and noncompetitive serotonin (5-hydroxytryptamine) antagonists have been uncovered over the past several years.[1] Interest in and the search for such antagonists are stimulated by reports that serotonin may be implicated in regulation of blood pressure, in renal regulation of water balance, in gastrointestinal motility, in malignant carcinoids, in asthma and allergy, as a secretory inhibitory hormone, and as a neurohumoral agent in the brain.

Approximately 450 compounds were screened for antiserotonin activity in the course of our present studies on various chemicals as possible antagonists for this amine. Included among these compounds were the antihistaminic agents. These studies showed that chlorcyclizine (Di-Paralene*), a piperazine type of antihistaminic, had good antiserotonin activity, both in vitro and in vivo. On the basis of these results, twenty-four analogues and homologues of this compound were tested for antiserotonin activity.

This report presents some of our findings on one such homologue, N-p-chloro-benzhydryl-N′-methyl homopiperazine dihydrochloride (SA-97), found to be an active serotonin antagonist (Fig. 1).

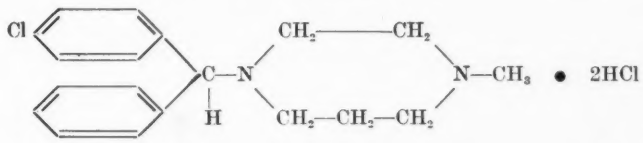

Fig. 1.—N-p-chloro-benzhydryl-N′-methyl-homopiperazine dihydrochloride (homochlorcyclizine; SA-97).

This compound† (synthesized by Sommers, Michaels, and Weston‡ of the Research Area, Abbott Laboratories) is a white powder which is soluble in water to more than 10 per cent; it is stable, with a sharp taste. The molecular weight of the salt is 387.78. Its generic name is homochlorcyclizine.

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*Di-Paralene is a trademark applied to chlorcyclizine by Abbott Laboratories.

†U. S. Patent 2,655,498.

‡Sommers, A. H., Michaels, R. J., and Weston, A. W.: J. Am. Chem. Soc. 76: 5805, 1954.

TABLE I. COMPARATIVE SPASMOLYTIC ACTIVITY OF HOMOCHLORCYCLIZINE (SA-97) AND CHLOROCYCLIZINE (DI-PARALENE)

TEST	HOMOCHLORCYCLIZINE (SA-97)		CHLORCYCLIZINE (DI-PARALENE)		REMARKS
	DOSE	RESPONSE	DOSE	RESPONSE	
Rat uterus	0.016 μg/ml.	50% inhibition of serotonin			Approximately twice as active as Chlorcyclizine; equal to BAS*
	0.025 μg/ml.	80% inhibition of serotonin	0.025. μg/ml.	35% inhibition of serotonin	
	0.05 μg/ml.	100% inhibition of serotonin			
Rat paw	10 mg./Kg. I.V.	Marked antagonism	10 mg./Kg. I.V.	None to mild antagonism	More active than Chlorcyclizine
	10-15 mg./Kg. I.P.	Marked antagonism			
Guinea pig aerosol	10 mg./Kg. I.P.	Mild protection vs. serotonin	10 mg./Kg. I.P.	No protection	More active than Chlorcyclizine
	20 mg./Kg. I.P.	Full protection vs. serotonin	15 mg./Kg. I.P.	Mild protection	
Antidiarrhea	16 mg./Kg. I.P.	50% protection vs. serotonin			Equal to BAS
Guinea pig ileum	0.5 μg/ml.	50% inhibition of serotonin	0.5 μg/ml.	10% inhibition of serotonin	2-5 times as active as Chlorcyclizine; equal to Papaverine, following $BaCl_2$; 1/30-1/36 atropinic activity
	0.025 μg/ml.	85% inhibition of histamine	0.05 μg/ml.	85% inhibition of histamine	
Guinea pig chain	1 μg/ml.	80% inhibition of serotonin	1 μg/ml.	47% inhibition of serotonin	Approximately twice as potent as Chlorcyclizine
	2.5 μg/ml.	27% inhibition of acetylcholine	2.5 μg/ml.	0% inhibition of acetylcholine	
	2.5 μg/ml.	100% blockade of histamine	2.5 μg/ml.	75% inhibition of histamine	
	50 μg/ml.	9 mm. relaxation of chain	50 μg/ml.	No relaxation of chain	
	100 μg/ml.	15 mm. relaxation of chain	100 μg/ml.	8 mm. relaxation	
Rabbit ileum		1/40-1/60 atropinic activity 1.75-2.5 X papaverine		1/100 atropinic activity	Approximately twice the atropinic activity of Chlorcyclizine
SRS-A	4×10^{-6} g/ml.	Antagonism (partial)			
Active anaphylaxis	15 mg./Kg. I.P.	Full protection	15 mg./Kg. I.P.	Full protection	Iso-active
Passive anaphylaxis	10 mg./Kg. I.P.	Full protection	10 mg./Kg. I.P.	Full protection	Iso-active
IV LD_{100} histamine in guinea pig	5 mg./Kg. P.O.	Full protection	5 mg./Kg. P.O.	Full protection	Iso-active

*1-Benzyl-2, 5 dimethyl serotonin.

METHODS

Antiserotonin activity was screened initially against serotonin-induced spasms in the isolated rat uterus preparation.[2] Those agents showing activity by this in vitro method were further tested in vivo against serotonin-evoked edema in the rat paw[3] and against bronchospasm induced in guinea pigs by an aerosol of serotonin.[4] Antianaphylactic activity was assessed in both egg-white-sensitized guinea pigs exposed to egg white aerosol and passively sensitized guinea pigs challenged intravenously with egg white. The Magnus method was used to test the effects of SA-97 on smooth muscle, while the guinea pig tracheal chain[5] was used to test its effects as a bronchodilator. Studies on the direct cardiac effects of the drug were carried out with Langendorff cat and monkey hearts perfused at a pressure head of 40 to 50 cm. with Locke's solution. Coronary flow studies were carried out in dogs with a Shipley-Wilson rotameter. Antipyretic activity was determined in normal rats and in those in which fever had been induced with yeast. The effect of SA-97 on monoamine oxidase activity was determined by both the manometric method[6] and the pigment test.[7] Analgesic activity was assessed by the method of Hardy, Wolff, and Goodell[8] as modified by Andrews and Workman.[9] Antiemetic activity was determined in dogs against intravenously administered apomorphine-induced emesis. Albino rabbits were used to assess the local irritant activity of SA-97 following intramuscular injection and instillation into the conjunctival sacs. The effect of SA-97 on the nictitating membrane responses of the cat, as triggered both electrically and by epinephrine, were recorded kymographically via direct mechanical means. The method of Toman and associates[10] was used to test the effect of the drug on electroshock seizure latency. Acute toxicity studies were carried out in mice, rats, guinea pigs, rabbits, cats, and dogs. The LD_{50} and its associated confidence limits were calculated according to the method of Litchfield and Wilcoxon.[11] Circulatory, respiratory, electrocardiographic, and other studies were performed in both anesthetized and unanesthetized animals in the usual manner.

RESULTS

Our initial rat uterus studies with chlorcyclizine showed this compound to have antiserotonin activity comparable to that of promethazine (Phenergan*) and one-half that of d-lysergic acid diethylamide (LSD). Among the analogues and homologues of chlorcyclizine, SA-97 proved to be the most active. It was found to gain in antiserotonin, antihistaminic, and antiacetylcholine potencies. SA-97 at a concentration of 0.05 μg per milliliter caused complete and prolonged inhibition of serotonin-induced spasms of the rat uterus preparation (Table I). An intravenous dose of 10 to 15 mg. SA-97 per kilogram showed marked antiserotonin activity in the rat paw preparation. One-hour pretreatment with an intraperitoneal dose of 20 mg. of the drug per kilogram afforded full protection to guinea pigs exposed for ten minutes to an aerosol of 0.5 per cent serotonin.

*Phenergan is a trademark applied to promethazine by Wyeth, Inc.

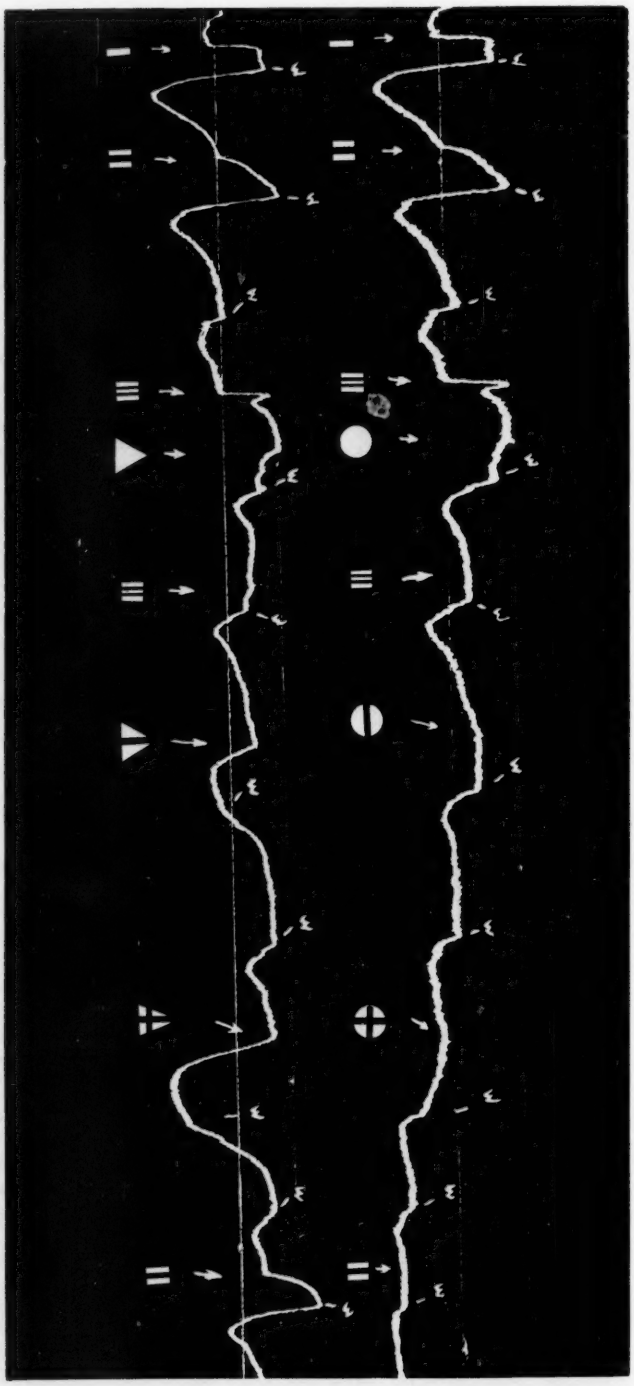


Fig. 2.—Comparative spasmolytic activity of aminophylline and SA-97 on the guinea pig tracheal chain sensitized to horse serum.

Key to Symbols: I = Acetylcholine chloride, 0.5 μg/ml.; *II* = histamine diphosphate, 2 μg/ml.; *III* = horse serum, 1:1,000; *circle* = SA-97, 10 μg/ml.; *barred circle* = SA-97, 25 μg/ml.; *crossed circle* = SA-97, 50 μg/ml.; *triangle* = aminophylline, 5 μg/ml.; *barred triangle* = aminophylline, 10 μg/ml.; *crossed triangle* = aminophylline, 25 μg/ml.

A 2.5 μg per milliliter dose of SA-97 did not inhibit serotonin-induced spasms of the rabbit ileum; in the guinea pig, however, 0.5 μg per milliliter caused approximately a 50 per cent inhibition of the contractions. This antagonism was approximately five times more active than that induced by chlorcyclizine. At concentrations of 0.5 to 1 μg per milliliter, it caused approximately a 75 per cent inhibition of serotonin-induced spasms in the guinea pig tracheal chain preparation, being roughly 1.5 times more potent than chlorcyclizine. Its atropinic activity on the ileum and chain was 2 and 2.5 times that of chlorcyclizine, respectively.

SA-97 was assayed by D. W. Woolley[12] of the Rockefeller Institute for Medical Research to be equal in antiserotonin potency to BAS (1-benzyl-2,5-dimethyl serotonin), both in vitro by the rat uterus method and in vivo by his antidiarrhea test. Central stimulation in mice with SA-97 was also noted, this being in marked contrast with the depression induced by BAS.

A 15 mg. per kilogram intraperitoneal dose of SA-97 afforded full protection against egg white aerosol in guinea pigs sensitized to egg white. A 10 mg. per kilogram dose, intraperitoneally, protected passively sensitized pigs against an intravenous challenge.

SA-97 was tested for its spasmolytic activity against contractions induced in the isolated guinea pig ileum by the slow-reacting substance SRS-A.* Preliminary experiments by W. E. Brocklehurst of the National Institute for Medical Research of England showed that SA-97 in a concentration of 4×10^{-6} G./ml. was able to reduce the spasmogenic activity of SRS-A by a factor of 3 or 4. This antagonism was not immediate but reached its maximum after SA-97 had been in contact with the tissue continuously for a period of ten to fifteen minutes.

Direct muscle-relaxant properties were demonstrated when 50 μg of the drug per milliliter caused relaxation of the tracheal chain musculature per se. It also showed 1.75 to 2.5 times the activity of papaverine on the rabbit ileum; it was equal in potency to papaverine when tested following barium on the guinea pig ileum.

Fig. 2 shows the comparative effects of SA-97 and aminophylline on spasms induced by horse serum on the tracheal chain preparation. These chains were prepared from guinea pigs sensitized to the antigen seventeen days previously with three sensitizing injections given on alternating days. Therapeutically, aminophylline was approximately twice as potent as SA-97 in directly relieving the antigen-induced spasm following a latent period of several minutes. This relaxation, however, was not always sustained, and on occasion it appeared to wear off. Washing the tissues readily reversed the relaxant action of aminophylline. Thus, the spasmolytic action of aminophylline was immediate, that is, rapid but comparatively transient.

SA-97, on the other hand, showed initially a slight but progressive relaxation of the spasm within the first ten to fifteen minutes. In contrast with aminophylline, washing the SA-97-treated tissues caused progressive relaxation of the chain with each washout, so that each washing caused a further

*Brocklehurst, W. E.: Ciba Foundation Symposium on Histamine, London, 1956, J. & A. Churchill, Ltd., pp. 175-179.

relaxation of the chain to near base line, that is, a reversed treppe effect. Thus, by contrast, the spasmolytic action of SA-97 was not immediate in action, like aminophylline, but was progressive and tenacious.

It is interesting to speculate whether the spasmolytic action of SA-97 on the tracheal chain preparation might perhaps be due in part to its ability to unite with the spasmogen to form a "complex." Progressive washout procedures could then remove increasing amounts of this "spasmogen-plus-SA-97" complex and thus relieve the tracheal spasm.

Langendorff cat heart preparations showed that 10 μg of SA-97 per milliliter increased the flow rate from about 10 to 80 per cent; 4 μg per milliliter caused an 18 per cent increase in flow in the monkey heart, together with a decrease in amplitude. Direct cannulation of the left carotid artery showed that SA-97 in a dose of 50 μg into the coronary circulation was as effective as a like dose of papaverine in producing a marked increase in flow. SA-97 in a 1 mg. per kilogram intravenous dose showed protection against aconitine-induced fibrillation in the intact dog heart.

An intravenous dose of 0.5 mg. SA-97 per kilogram did not cause any circulatory fluctuations in an anesthetized dog. Doses of 1 and 2 mg. per kilogram caused transient 10 and 30 mm. falls in the blood pressure. Electrocardiographic studies in unanesthetized dogs medicated intravenously with 1, 2, and 5 mg. of SA-97 per kilogram showed a temporary heightening of the ST segment and a transient increase in pulse rates at the 5 mg. per kilogram dose. No other abnormalities were seen.

Intravenous doses of 1 to 4 mg. of SA-97 per kilogram generally induced transient 20 to 50 mm. drops in the blood pressure of anesthetized cats. These doses, in addition, caused 20 to 75 per cent inhibition of serotonin on the blood pressure. Pressor effects were changed to depressor effects. Epinephrine responses following SA-97 were equivocal. A 1 mg. per kilogram intravenous dose of this antiserotonin agent blocked the electrically stimulated nictitating membrane responses approximately 20 per cent. Complete inhibition of these responses occurred with a 10 mg. per kilogram dose. A 0.25 mg. per kilogram intravenous dose per se had no effect on the nictitating membrane; relaxation occurred in two out of five cats at 1 mg. per kilogram.

An oral dose of 100 mg. of this serotonin antagonist per kilogram lowered the body temperature of mice approximately 1.6° C. Rectal temperatures of rats on chronic toxicity at oral doses of 20 and 30 mg. per kilogram were 0.5 to 2.0° C. higher than controls. Rectal temperatures of dogs on chronic toxicity with SA-97 for over fifteen months at a dose level of 15 mg. per kilogram, orally, were substantially the same as controls, being about 0.5° F. lower over an eight-hour observation period. SA-97 showed weak inhibition of monoamine oxidase activity when tested by the manometric method with Tyramine hydrochloride as the substrate. A 1 by 10^{-4} M concentration inhibited monoamine oxidase from rat liver mitochondria only 13 per cent. By comparison, iproniazid (Marsilid*) at 1 by 10^{-5} M showed approximately

*Marsilid is a trademark applied to iproniazid by Hoffmann-La Roche, Inc.

a 50 per cent inhibition. Inhibition of monoamine oxidase by SA-97 could not be detected by the pigment test with serotonin creatinine sulfate as the substrate.

SA-97 had no local anesthetic activity when tested on the corneal surfaces of guinea pigs. It failed to show any antagonistic activity against morphine toxicity in mice. There was no significant effect on electroshock seizure threshold latency. An oral dose of 25 mg. SA-97 per kilogram was able to confer moderate antipyretic activity against yeast-induced fever in rats. No analgesic activity was seen in dogs pretreated with 10 mg. per kilogram of this serotonin blocker given intravenously and exposed to a radiant stimulus. SA-97 showed weak antiemetic activity against intravenous apomorphine in dogs; only at relatively high doses of 32 and 64 mg. per kilogram, subcutaneously, did SA-97 show any such action.

In marked contrast with most serotonin antagonists that we tested was the finding that animals treated with SA-97 showed excitation rather than sedation as the principal symptom. Animals on acute oral toxicity with the drug (Table II) showed secondary excitation which lasted twenty-four to forty-eight hours following an initial period of depression. This biphasic effect was not as pronounced with intravenous injection, the primary depressant phase being overshadowed by the excitant phase. Thus, mice pretreated orally

TABLE II. ACUTE TOXICITY OF HOMOCHLORCYCLIZINE (SA-97)

SPECIES	ROUTE	SYMPTOMATOLOGY	LD_{50} AND 95 PER CENT CONFIDENCE LIMITS (MG./KG.)
Mice	P.O.	Initial sedation, secondary excitation; ataxia, hypersensitivity; clonic-tonic convulsions	390 (336-452)
	I.V.	Tremors, ataxia, jerks, tonic-clonic convulsions	47 (45- 49)
	I.P.	Excitation, jerks, clonic-tonic convulsions	125 (106-145)
	S.C.	Excitation, jerks, convulsions	135 (115-158)
Rats	P.O.	Rapid respiration, excitation	490 (452-532)
	I.V.	Rapid respiration, tremors, excitation, ataxia, convulsive death	36 (34- 38)
	I.P.	Jerks, tremors, excitation, clonic convulsions	80 (70- 92)
Guinea Pigs	P.O.	Initial depression, secondary excitation; jerks, ataxia; clonic-convulsive death	390 (368-414)
	I.V.	Rapid respiration, tremors, ataxia, convulsive death	19 (17- 22)
	I.P.	Rapid respiration, depression, ataxia, jerks, tremors, clonic-tonic convulsions	76 (63- 91)
Rabbits	I.V.	Rapid respiration, tremors, jerks, convulsive death	20-25*
Cats	I.P.	Tremors, jerks, clonic convulsions, prolonged pupillary dilatation	50-75†
Dogs	P.O.	Emesis, slight depression, excitation, jerks, slight apprehension	> 200‡
	I.P.	Emesis, excitation, jerks, tremors, clonic convulsions	50-75§
	I.V.	Retching, ataxia, tremors, emesis, clonic-tonic convulsions	20-30‖

*Between 20 and 25 mg. per kilogram, three animals per dose level.
†Between 50 and 75 mg. per kilogram, two animals per dose level.
‡More than 200 mg. per kilogram; doses from 50 to 200 all cause emesis with drug loss.
§Between 50 and 75 mg. per kilogram, two animals per dose level.
‖Between 20 and 30 mg. per kilogram, three animals per dose level.

with 50 mg. of SA-97 per kilogram daily for five days showed initial prolongation of pentobarbital sodium sleep times, followed later, upon retesting with the barbiturate, by a marked shortening of the sleep times. This secondary antagonism toward the barbiturate lasted approximately three weeks under the conditions of the experiment.

Chronic toxicity studies have been in progress for about one year in dogs at dose levels of 10 and 15 mg. per kilogram and in rats at doses of 10, 20, 30, and 60 mg. per kilogram orally. Gross and histologic examination of animals after three to sixteen months' medication showed no morphologic changes that could be attributed to drug action.*

DISCUSSION

Hawkins,[13] in his studies on the bronchoconstrictor and bronchodilator actions of twelve well-known antihistaminic drugs, reported that all showed constrictor action when tested on the guinea pig tracheal chain in concentrations greater than 10^{-6}. Higher doses of these compounds, from 10^{-4} to 10^{-3}, caused relaxation. This constrictor action was reported to be the least marked and the dilator action most pronounced with antazoline (Antistine†) and chlorcyclizine (Di-Paralene). Of interest was the observation that the structural feature not possessed by the others tested which these two drugs had in common was the inclusion of the "aliphatic nitrogen" in a heterocyclic ring. The imidazoline ring was present in the antazoline molecule, and in chlorcyclizine the piperazine ring was present. By contrast, the basic structure of the other antihistaminics of the ethylenediamine series consisted of aromatic or heterocyclic rings attached to one of the nitrogen atoms, while the other nitrogen atom was generally substituted by aliphatic groups. Our studies on SA-97 were an outgrowth of this report by Hawkins.

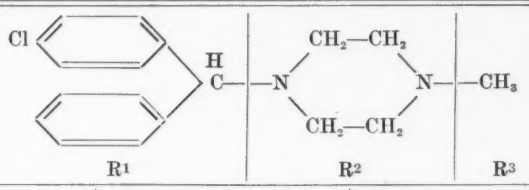
The following conclusions can be gleaned from the data presented in Table III depicting the comparative antiserotonin activity of compounds related to chlorcyclizine and SA-97: (1) quaternization decreases antiserotonin activity; (2) increasing or decreasing the length of the side chain attached to the terminal nitrogen (methyl group in position R^3) decreases antiserotonin activity; (3) inclusion of another CH_2 grouping in the six-membered piperazine ring to form the seven-membered homopiperazine ring increases antiserotonin activity; (4) the addition of a methyl group to either the piperazine or the homopiperazine ring and the addition of a keto group to the homopiperazine ring decrease activity; (5) substitution of the benzhydryl group in place of the p-chloro-benzhydryl group decreases the activity in both the piperazine and the homopiperazine series; (6) replacement of the chlorine atom with a methoxy group decreases antiserotonin potency; and (7) replacement of the p-chloro-benzhydryl group by a fluorene group destroys activity.

Of interest is the finding that SA-97 is able to antagonize the slow-reacting substance (SRS-A). This spasmogen, probably an unsaturated fatty acid,

*Histopathologic examinations were carried out in the Pathology Section under the direction of Dr. R. J. Stein.

†Antistine is a trademark applied to antazoline by Ciba Pharmaceutical Products, Inc.

TABLE III. COMPARATIVE ANTISEROTONIN ACTIVITY OF CHLORCYCLIZINE (DI-PARALENE) ANALOGUES AND HOMOLOGUES AS TESTED ON THE RAT UTERUS PREPARATION



SA #	R1	R2	R3	ANTI-SEROTONIN POTENCY*
2	Unchlorinated	—	—	40
21	CH_3 in place of Cl	—	—	80
52	—	—	CH_2-thienyl	60
77	—	—	$(CH_2)_2$—O—$(CH_2)_2$—OH	50
83	Meta-chloro	—	—	80
84	Ortho-chloro	—	—	60
87	—	—	H	40
90	—	—	—	80
91	Unchlorinated	Homopiperazine	—	70
92	Fluorene	—	$(CH_2)_2$—OH	0
97	—	Homopiperazine	—	100
99	$(C_6H_5)_2$—CH—CH_2	—	—	70
100	CH_3O in place of Cl	—	—	20
107	—	—	$(CH_2)_2$—OH	50
120	Br in place of Cl	—	—	90
150	—	CH_3 on piperazine	—	60
153	Unchlorinated	—	CH_2—C_6H_4—O—CH_3	40
257	$(C_6H_5)_2$—C—OH—CH_2	CH_3 on piperazine	$(CH_3)_2(+)CH_3SO_4(-)$	0
355	—	3-Keto homopiperazine	H	20
367	—	—	H	40
385	—	4,6-(CH_3)2-homopiperazine	—	80
400	H	Homopiperazine	CHO	0
425	—	Homopiperazine	Cyclopropyl	20
434	—	Homopiperazine	$(CH_2)_2$—OH	60
441	—	Homopiperazine	(CH_2)—H—$(C_2H_5)_2$	20

*SA-97 given an arbitrary value of 100.

is released from the lungs of sensitized guinea pigs in anaphylactic shock when the lungs are perfused with a solution of the antigen.[14] It is not detected in the perfusate or in extracts from sensitized lungs without contact with the antigen.

Of clinical interest is the finding that the slow-reacting substance appears also in the effluent of human asthmatic lungs perfused with allergen solution. Both this substance and the SRS-A from guinea pigs appear to be identical. The slow-reacting substance from human asthmatic lungs is reported capable of causing bronchoconstriction.

SA-97 is reported chiefly as an antiserotonin agent because of the great potency in this connection and the considerable interest in drugs having such properties. We feel that the outstanding features are its marked antiserotonin effect and its potent antihistaminic and significant anticholinergic actions. We doubt that these factors alone would explain its apparent clinical usefulness in the management of chronic asthma, which is being reported separately.*

*Fisherman, E. W., Feinberg, S. M., Feinberg, A. R., and Pruzansky, J. J.: Homochlorcyclizine: an antiallergic drug with multiple clinical properties, J. ALLERGY, 31: 232, 1960.

It is possible that the activity against the slow-reacting substance of Brocklehurst can account, at least in part, for its antiasthmatic properties.

We consider it quite possible that on further study SA-97 may show antagonistic effects against other substances of known or unknown nature which may be involved in certain allergic or nonallergic manifestations.

Work of this type is in progress.

SUMMARY

1. N-p-chloro-benzhydryl-N′-methyl homopiperazine dihydrochloride (homochlorcyclizine; SA-97) was found to be an active serotonin antagonist, both in vitro and in vivo.

2. Homochlorcyclizine further showed a blocking action against histamine and acetylcholine.

3. In addition, homochlorcyclizine was able to antagonize the spasmogenic action of the slow-reacting substance (SRS-A), an agent thought to be implicated as a spasmogen in both anaphylactic shock and human asthma.

4. Homochlorcyclizine showed a relaxant action on the guinea pig tracheal chain preparation *sui generis* as well as against contractions induced by serotonin, histamine, acetylcholine, and horse serum.

5. The compound shielded animals against both passive and active anaphylaxis.

6. Homochlorcyclizine showed a coronary dilator action as well as antifibrillatory properties.

7. A biphasic depressant-stimulant action on the central nervous systems of animals was noted, both acutely and chronically.

8. Homochlorcyclizine showed weak activity or no activity in tests for antipyresis, analgesia, antiemesis, and monoamine oxidase inhibition.

9. The compound was tested for its acute toxicity in mice, rats, guinea pigs, rabbits, cats, and dogs.

10. The clinicopathologic and histopathologic findings in both rats and dogs medicated with the drug for over one year showed no morphologic changes which could be attributed to drug action.

11. Homochlorcyclizine's suggested therapeutic use in the field of allergy is based on both its bronchodilator activity and its spasmolytic action toward serotonin, histamine, acetylcholine, and the slow-reacting substance (SRS-A), coupled with its protective action against passive and active anaphylaxis.

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REFERENCES

1. Page, I. H.: Serotonin (5-Hydroxytryptamine); the Last Four Years, Physiol. Rev. **38**: 277, 1958.
2. Costa, E.: Effects of Hallucinogenic and Tranquilizing Drugs on Serotonin Evoked Uterine Contractions, Proc. Soc. Exper. Biol. & Med. **91**: 39, 1956.
3. Benditt, E. P., and Rowley, D. A.: Antagonism of 5-Hydroxytryptamine by Chlorpromazine, Science **123**: 24, 1956.
4. Herxheimer, H.: The 5-Hydroxytryptamine Shock in the Guinea-Pig, J. Physiol. **128**: 435, 1955.

5. Castillo, J. C., and de Beer, E. J.: The Tracheal Chain. I. A Preparation for the Study of Antispasmodics With Particular Reference to Bronchodilator Drugs, J. Pharmacol. & Exper. Therap. **90**: 104, 1947.
6. Zeller, E. A., and Barsky, J.: In vivo Inhibition of Liver and Brain Monoamine Oxidase by 1-Isonicotinyl-2-isopropylhydrazine, Proc. Soc. Exper. Biol. & Med. **81**: 459, 1952.
7. Wykes, A. A., Gladish, Y. C., and Taylor, J. D.: A Screening Method for the Rapid Detection of Monoamine Oxidase Inhibitors In Vitro, Fed. Proc. **18**: 462, 1959.
8. Hardy, J. D., Wolff, H. G., and Goodell, H.: Studies on Pain: A New Method for Measuring Pain Threshold; Observations on Spatial Summation of Pain, J. Clin. Invest. **19**: 649, 1940.
9. Andrews, H. L., and Workman, W.: Pain Threshold Measurements in the Dog, J. Pharmacol. & Exper. Therap. **73**: 99, 1941.
10. Toman, J. E. P., Everett, G. M., and Smith, A. H., Jr.: Use of Electroshock Seizure Latency for Pharmacological Testing in Mice, Fed. Proc. **14**: 391, 1955.
11. Litchfield, J. T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharmacol. & Exper. Therap. **96**: 99, 1949.
12. Woolley, D. W.: Convenient Method for Assay In Vivo of Antimetabolites of Serotonin, Proc. Soc. Exper. Biol. & Med. **98**: 367, 1958.
13. Hawkins, D. F.: Bronchoconstrictor and Bronchodilator Actions of Antihistamine Drugs, Brit. J. Pharmacol. **10**: 230, 1955.
14. Vogt, W.: Naturally Occurring Lipid-Soluble Acids of Pharmacological Interest, Pharmacol. Rev. **10**: 407, 1958.

SEROTONIN- AND HISTAMINE-AFFECTING AGENTS AND EXPERIMENTAL VASCULAR SENSITIZATION

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INTRODUCTION

In a previously reported study[1] it was noted that the administration of reserpine to rabbits subjected to active sensitization procedures resulted in a suppressive effect on the expected development of lesions of myocarditis and arteritis associated with this experimental procedure. Reports suggesting the etiological importance of serotonin released in a physiologically active state in rabbit anaphylaxis[2-4] led to a consideration of the tissue serotonin-releasing and -depleting action of reserpine as the possible modifying factor of histopathologic vascular response to in vivo antigen-antibody reaction. In further exploration of this situation, it was felt that a study of comparative actions of other serotonin-affecting drugs might be of importance in evaluating possible serotonin-vascular hypersensitivity relationships. This could not be accomplished in active sensitization experiments, however, since cumulative daily dosages of such pharmacologic agents prerequisite for effective and maintained states of serotonin depletion and/or neutralization in rabbits would result in toxic drug levels that would prevent their survival over the required eighteen-day experimental period. An experimental approach did become feasible when a procedure was evolved in this laboratory for the rapid and uniform development of histopathologic lesions of panarteritis, periarteritis, myocarditis, glomerulitis, and pulmonary eosinophilic granulomas over a forty-eight hour experimental period through the utilization of passive reversed subanaphylactic sensitization techniques.[5] This experimental procedure allowed for the simultaneous administration of serotonin-modifying agents, reserpine,[6-7] brom-lysergic acid diethylamide,[8-9] chlorpromazine,[10-11] promethazine,[12-14] hydroxyzine,[15-16] cyproheptadine,[17] and iproniazid,[18-19] to rabbits subjected to passive sensitization techniques favorable to the production of vascular sensitization. During the course of these studies experimental data suggested a possible etiological role for an associated pathophysiologic mechanism involving histamine action.

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The experimental plan was then extended to include a similar evaluation of antihistaminic drugs, tripelennamine and diphenhydramine.

EXPERIMENTAL PROCEDURE

Detailed instructions for the production of vascular lesions by passive reversed subanaphylactic sensitization in the rabbit are presented elsewhere.[5] Briefly, this procedure consists of the administration of 10 ml. of sterile bovine serum through the marginal ear vein. Twenty-four hours later serum is obtained from blood withdrawn through the central artery of the rabbit's ear to ascertain the presence of circulating bovine serum antigens, demonstrated by interfacial serum precipitin tests with homologous species-specific antiserum.

TABLE I

DRUG	DOSAGE	ROUTE OF ADMINISTRATION	TIMES OF ADMINISTRATION	
			PRECHALLENGE	POSTCHALLENGE
Reserpine (Serpasil, Ciba)	1 mg./kg. 0.2 mg./kg.	Subcutaneous Intravenous	24 hours 1 hour	
Brom-lysergic acid diethylamide (BOL-148, Sandoz)	100 mg./kg.	Intravenous	5 minutes	8-12 hours
Chlorpromazine (Thorazine, Smith Kline and French)	2.5 mg./kg.	Intravenous	5 minutes	8-12 hours
Hydroxyzine hydrochloride (Atarax, Roerig)	10 mg./kg.	Intravenous	5 minutes	8-12 hours
Promethazine (Phenergan, Wyeth)	1 mg./kg.	Intravenous	5 minutes	1 and 6 hours
Cyproheptadine (Merck)	0.15 mg./kg.	Intravenous	5 minutes	8-12 hours
Iproniazid (Marsilid, Hoffman-La Roche)	25 mg./kg.	Subcutaneous	24 hours and 1 hour	
Tripelennamine hydrochloride (Pyribenzamine, Ciba)	1 mg./kg.	Intravenous	5 minutes	1 and 6 hours
Diphenhydramine (Benadryl, Parke, Davis)	1 mg./kg.	Intravenous	5 minutes	1 and 6 hours
Chlorpromazine and tripelennamine	Combined administration of each drug as above			
Brom-lysergic acid diethylamide and diphenhydramine	Combined administration of each drug as above			

Each animal is then immediately subjected to passive reversed subacute anaphylactic challenge with high-titer homologous species antiserum containing antibodies to eight constituent bovine serum antigens. Five milliliters of such antiserum diluted in 45 ml. of physiologic saline is administered by slow intravenous drip through the marginal ear vein at a rate of approximately twenty

drops per minute, adjusted in each animal to avoid precipitating overt manifestations of anaphylaxis. Twenty-four hours later each animal is sacrificed by air embolism, and multiple tissue sections taken from the lungs, heart, and kidneys are prepared for histologic study.

Eighty-eight rabbits, ranging in weight from 2 to 2.5 kilograms, were employed in passive reversed subanaphylactic sensitization procedures. Eight of these rabbits served as controls, while the remaining eighty were divided into ten experimental groups. Each group consisted of eight animals given one particular pharmacologic agent in addition to the bovine serum and homologous antiserum. These experimental procedures, including dosages, routes, and times of administration, are given in Table I.

RESULTS

Detailed descriptions of the type of vascular lesions noted in rabbits subjected to passive reversed subanaphylactic sensitization techniques are given elsewhere.[5] Briefly, these are as follows: The most consistently observed lesions involve the small pulmonary arteries and arterioles which exhibit varying degrees of perivascular and adventitial infiltration, chiefly with eosinophilic granular cells and with much lesser numbers of polymorphonuclear leukocytes. Eosinophils are also found infiltrating the media and intima, and often adventitial and medial edema, early intimal proliferation, and intercellular disruption of the intimal lining are evident. Occasionally, infiltrations consisting of eosinophilic granular-staining cells with a few interspaced large mononuclear cells are sufficiently extensive to resemble discrete granulomas. Less commonly observed lesions include eosinophilic cellular infiltrations scattered throughout myocardial tissue and those of an early proliferative glomerulitis characterized by increased cellularity and basement membrane edema of some renal glomeruli.

All animals in the control and drug-treated groups exhibited evidence of scattered eosinophilic granular cellular infiltration throughout the myocardium. In the majority of rabbits in each group (six or seven out of every eight) there were demonstrated histologic changes of early proliferative glomerulitis without apparent differences on the basis of treatment. However, definite histopathologic differences could be noted among the lung sections of animals on the basis of involvement of the pulmonary vasculature. These varied from the typical lesions of arteritis described above (Fig. 1 *A* to *E*) to a complete absence of any vascular involvement in multiple sections of each lobe. Periarteritis was classified as mild or attenuated when only a few vessels were noted to be affected with a light cellular perivascular ringing in the absence of medial and/or intimal involvement (Fig. 1, *F*). A summary of these pulmonary vascular findings is given in Table II.

COMMENTS

That endogenous serotonin might be involved in pathophysiologic reactions resulting in hypersensitivity vasculitis has been discussed in an earlier report.[1]

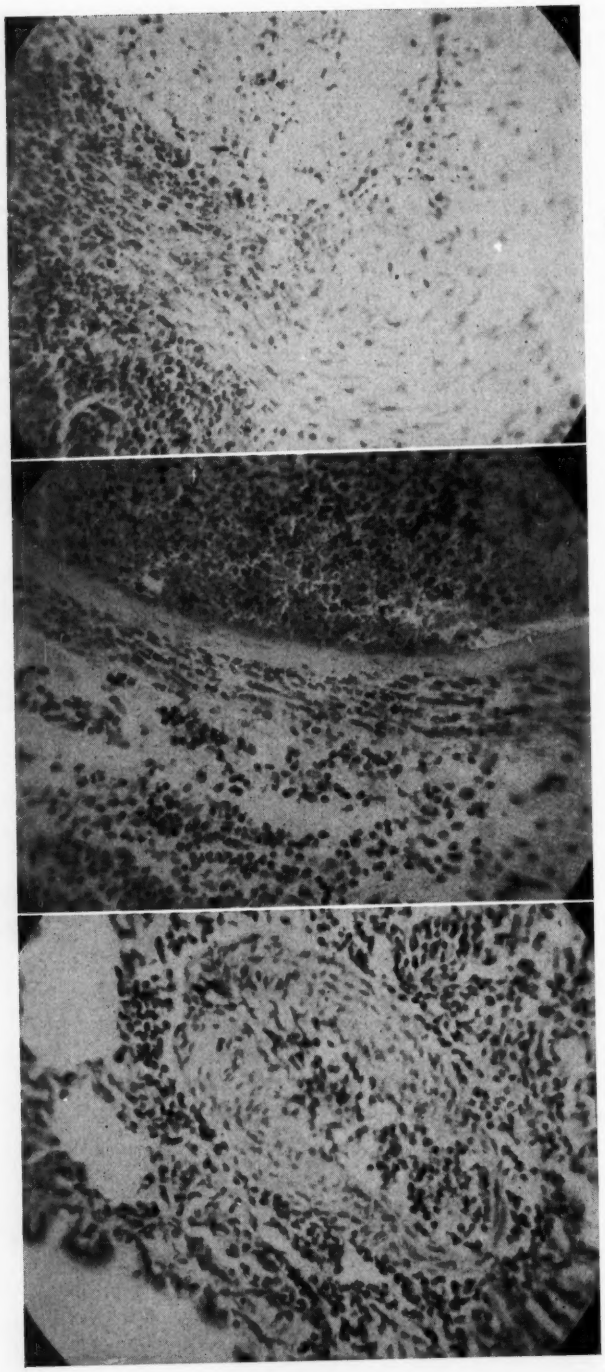
TABLE II

DRUG TREATMENT	NUMBER OF RABBITS EXHIBITING LESIONS	
	A. PULMONARY ARTERITIS IN EACH GROUP OF EIGHT	B. IN GROUP A OF ONLY ATTENUATED VASCULITIS
None	8	0/8
Reserpine	7	5/7
BOL-148	7	4/7
Chlorpromazine	7	3/7
Promethazine	2	1/2
Hydroxyzine	7	1/7
Cyproheptadine	3	2/3
Iproniazid	8	2/8
Tripelennamine	7	2/7
Diphenhydramine	8	2/8
Chlorpromazine and tripelennamine	2	1/2
BOL-148 and diphenhydramine	6	0/6

Briefly, evidence for this possibility is suggested by (1) blood serotonin levels reported in rabbit anaphylaxis;[2-4] (2) an evaluation of experimental conditions indicating the development of vasculitis in this species as a subacute manifestation of such anaphylactic reaction;[5, 20-22] (3) the suppressive effect of reserpine, a serotonin-releasing agent, on experimentally produced lesions of cardiovascular sensitization;[1] and (4) histopathologic alterations noted in arterial and arteriolar intima as a result of directly increasing in vivo serotonin beyond physiologic limits.[23] Thus, any histopathologic modifications evoked by serotonin-affecting mechanisms during the process of cardiovascular sensitization might offer further indirect evidence concerning its etiological role.

Passive sensitization techniques appeared to be well suited for this type of study. Control offered over the exact timing of in vivo antigen and antibody appearance allowed for the simultaneous introduction of pharmacologic effects at the suspected time of onset and height of immunologic reactivity and tissue response. Serotonin-modifying agents were thus employed in dosages previously determined to be mediated through (1) liberation and reduction in concentration of tissue serotonin (by reserpine);[6-7] (2) antagonism to the peripheral action of serotonin (by brom-lysergic acid diethylamide, chlorpromazine, promethazine, hydroxyzine, and cyproheptadine), possibly through an antimetabolite role competing for receptor sites;[24-25] and (3) potentiation of the action of serotonin (by iproniazid) through the inhibition of monamine oxidase, an enzyme which can inactivate serotonin.[18-19]

Because of the segmental nature and irregular distribution of the lesions of pulmonary arteritis, it is extremely difficult to quantitate them exactly. However, the study of multiple lung sections from each animal partially met this objection and proved important in establishing the pattern of histopathologic response. Thus, the group of untreated sensitized and challenged rabbits presented the uniform development of lesions of pulmonary arteritis and periarteritis, whereas the microscopic picture of those sensitized and simultaneously treated with antiserotonin agents was associated with varying degrees of histologic inhibition and/or attenuation of such vasculitis. The administration of



C.

B.

A.

D. *E.* *F.*

Fig. 1.—Lung sections from rabbits subjected to reversed passive subanaphylactic sensitization and challenge procedures with bovine serum antigens and homologous antisera without (*A*) and with (*B-F*) simultaneous drug treatment. In addition to bovine serum and homologous antiserum, rabbits *B* to *F* received other drugs, as follows: *B*, chlorpromazine; *C*, hydroxyzine; *D*, iproniazid; *E*, diphenhydramine; and *F*, cyproheptadine. Lesions of the pulmonary vasculature are shown to illustrate various stages of periarterial, adventitial, medial, and intimal cellular infiltration chiefly with eosinophilic granulocytes and lesser numbers of polymorphonuclear leukocytes. A thrombus containing large numbers of eosinophils is seen in *B*, reaction of early intimal hyperplasia in *A*, medial edema in *E*, and disruption of portions of the media in *C* and *D*. A very minimal and equivocal degree of tissue response is seen in *F*, where a small collection of eosinophilic granular cells can be found in the adventitia at the upper left-hand section of the artery.

promethazine and cyproheptadine was most marked in this regard: much lesser effects were noted with reserpine, chlorpromazine, and BOL; and little, if any, modification was noted with hydroxyzine. Allowing for prolonged duration of action of serotonin through inhibition of its enzymatic inactivation by iproniazid did not appear to enhance the degree of severity or intensity of arterial, myocardial, or glomerular lesions.

It is to be noted that the only definite supressively effective drugs, promethazine and cyproheptadine, are not only antiserotonin metabolites but are also known to possess marked antihistaminic activity. However, tripelennamine and diphenhydramine, potent antihistaminic agents without antiserotonin action,[12, 14] were ineffective in modifying the development of lesions of passive sensitization vasculitis. This finding paralleled the experience of others[26–28] studying antihistaminic agents in active sensitization procedures.

Consideration was then given to the possibility that modification in the action of both serotonin and histamine released during in vivo antigen-antibody reactions was required to attenuate the tissue responses resulting in lesions of vascular sensitization. This led to a study of the action of drug combinations in this regard. The antihistaminic, tripelennamine, and the antiserotonin agent, chlorpromazine, were each relatively ineffective in solo administration. Through their cumulative action, however, a suppressive and attenuating effect was noted on the development of pulmonary periarteritis and panarteritis to approximately the same degree as that demonstrated by promethazine or cyproheptadine. However, the combination of BOL and diphenhydramine did not enhance the resultant effect of either agent given alone. The in vivo action of BOL was disappointing in this regard, since lysergic acid diethylamide and derivatives are among the most potent of in vitro antiserotonin agents. Lack of in vivo antiserotonin vascular symptomatic effect has also been reported in studies of human patients with the carcinoid syndrome.[29]

The exact significance of these findings cannot be definitely established. Within the limits of these experimental data, however, it is suggested that the development of hypersensitivity vasculitis in the rabbit may be dependent upon multiple pathophysiologic processes, including both serotonin and histamine release. Modification of the in vivo role of both, rather than of either agent alone, appears to be important for an attenuating effect on the arterial tissue response to such immunologic-sensitizing phenomena.

SUMMARY

1. Nine serotonin- and histamine-affecting drugs were studied for an evaluation of their effects upon the development of lesions of hypersensitivity vasculitis produced in rabbits by passive reversed subanaphylactic sensitization techniques with bovine serum antigens.

2. Only promethazine and cyproheptadine, with both potent antiserotonin and antihistamine actions, and the combination of chlorpromazine and tripelennamine were effective in suppressing and attenuating microscopic lesions of pulmonary periarteritis and panarteritis.

3. Those pharmacologic agents whose actions include serotonin antagonism (brom-lysergic diethylamide, chlorpromazine, and hydroxyzine), serotonin release (reserpine), serotonin potentiation (iproniazid), and antihistaminics (tripelennamine and diphenhydramine) were relatively ineffective within the limits of this experimental procedure.

4. It is suggested that the development of hypersensitivity vasculitis in the rabbit may be dependent upon multiple pathophysiologic processes, including those associated with tissue response to both serotonin and histamine release.

REFERENCES

1. Cohen, S. G., and Mines, S. C.: Reserpine Effects and Experimentally Produced Lesions of Cardiovascular Sensitization and Eosinophilia, J. ALLERGY **30**: 169, 1959.
2. Humphrey, J. H., and Jaques, R.: The Release of Histamine and 5-Hydroxytryptamine (Serotonin) From Platelets by Antigen-Antibody Reaction (In Vitro), J. Physiol. **128**: 9, 1955.
3. Waalkes, T. P., Weissbach, H., Bozicevich, J., and Udenfriend, S.: Serotonin and Histamine Release During Anaphylaxis in the Rabbit, J. Clin. Invest. **36**: 1115, 1957.
4. Waalkes, T. P., Weissbach, H., Bozicevich, J., and Udenfriend S.: Further Studies on Release of Serotonin and Histamine During Anaphylaxis in the Rabbit, Proc. Soc. Exper. Biol. & Med. **95**: 479, 1957.
5. Cohen, S. G., and Sapp, T. M.: Histopathologic Cardiovascular Responses to Passive Sensitization, J. Infect. Dis. **105**: 124, 1959.
6. Pletscher, A., Shore, P. A., and Brodie, B. B.: Serotonin Release as Possible Mechanism of Reserpine Action, Science **122**: 374, 1955.
7. Passonen, M. K., and Vogt, M.: The Effects of Drugs on the Amounts of Substance P and 5-Hydroxytryptamine in Mammalian Brain, J. Physiol. **131**: 617, 1956.
8. Cerletti, A., and Rothlin, E.: Role of 5-Hydroxytryptamine in Mental Diseases and Its Antagonism to Lysergic Acid Derivatives, Nature **176**: 785, 1955.
9. Rothlin, E.: LSD and Related Substances, Ann. New York Acad. Sc. **66**: 668, 1956.
10. Costa, E.: The Effects of Hallucinogenic and Tranquilizing Drugs on the Serotonin Evoked Uterine Contractions, Proc. Soc. Exper. Biol. & Med. **91**: 39, 1956.
11. Himwich, H. E.: Psychopharmacologic Drugs, Science **127**: 59, 1958.
12. Doepfner, W., and Cerletti, A.: Über den Serotonin-Antagonismus einiger Antihistaminika unter Berücksichtigung ihrer Chemischen Struktur, Internat. Arch. Allergy **10**: 348, 1957.
13. Rose, J. C.: Pulmonary Vascular Responses to Serotonin and the Effects of Serotonin Antagonists, J. Clin. Invest. **36**: 924, 1957.
14. Doepfner, W., and Cerletti, A.: Comparison of Lysergic Acid Derivatives and Antihistamines as Inhibitors of the Edema Provoked in the Rat's Paw by Serotonin, Internat. Arch. Allergy **13**: 89, 1958.
15. Levi, S., Preat, S., Beersaerts, J., Dauby, J., Beelen, L., and Baugniet, V.: A Pharmacological Study of Hydroxyzine or U.C.B. 492, a Bisubstituted Derivative of Piperazine, Arch. internat. de pharmacodyn. et de thérap. **119**: 127, 1957.
16. Feinberg, A. R., Pruzansky, J. J., Feinberg, S. M., and Fisherman, E. W.: Hydroxyzine (Atarax) in Chronic Urticaria and in Allergic Manifestations, J. ALLERGY **29**: 358, 1958.
17. Merck Institute for Clinical Research: Cyproheptadine, Preclinical Evaluation, December, 1958.
18. Spector, S., Prockop, D., Shore, P. A., and Brodie, B. B.: Effect of Iproniazid on Brain Levels of Norepinephrine and Serotonin, Science **127**: 704, 1958.
19. Pletscher, A., and Gey, K. F.: Sterospecificity of Monoamine Oxidase Inhibitors, Science **128**: 900, 1958.
20. Cohen, S. G., Mayer, L. D., and Criep, L. H.: The Experimental Production of Arteritis by Passive Sensitization, J. Immunol. **66**: 487, 1951.
21. Cohen, S. G., Mokychic, W. E., and Drapiewski, J. F.: The Etiological Role of Streptococcus Toxin-Antitoxin Systems in the Experimental Production of Arterial Sensitization, J. ALLERGY **24**: 411, 1953.
22. Cohen, S. G., Dzury, D. S., and Michelini, F. J.: Staphylococcus Aureus Antigens in Hypersensitivity Reactions and Experimental Arterial Sensitization, J. ALLERGY **28**: 331, 1957.
23. Rossi, P., and Zamboni, L.: Pulmonary Arteriosclerosis Due to Serotonin, Nature **181**: 1216, 1958.

24. Wooley, D. W., and Shaw, E.: Antimetabolites of Serotonin, J. Biol. Chem. **203**: 69, 1953.
25. Wooley, D. W., and Shaw, E.: A Biochemical and Pharmacological Suggestion About Certain Mental Disorders, Science **119**: 587, 1954.
26. MacGregor, A. G., and Wood, D. R.: The Effect of Neoantergan and of Benadryl on Serum-Induced Myocarditis in Rabbits, Brit. J. Pharmacol. & Chemotherap. **4**: 216, 1949.
27. Dammin, G. J., and Bukantz, S. C.: Modification of Biologic Response in Experimental Hypersensitivity, J. A. M. A. **139**: 358, 1949.
28. Roberts, R. C., Crockett, K. A., and Laipply, T. C.: Effects of Salicylates and Benadryl on Experimental Anaphylactic Hypersensitive Vascular and Cardiac Lesions, Arch. Int. Med. 84: 48, 1949.
29. Schneckloth, R., Page, I. H., del Greco, F., and Corcoran, A. C.: Effects of Serotonin Antagonists in Normal Subjects and Patients With Carcinoid Tumors, Circulation **16**: 523, 1957.

SERUM SICKNESS

Serologic Studies Following Prophylactic Tetanus Antitoxin

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SINCE publication of the monumental treatise on serum disease by Von Pirquet and Schick,[1] innumerable investigators have reported on the various facets of this condition during the past fifty years. It is the purpose of this presentation to report on the antibody response in human beings following the injection of small prophylactic amounts of tetanus antitoxin.

Early investigators[2-6] have demonstrated that the incidence of serum sickness is proportional to the amount of horse serum injected. If 100 c.c. of "native" horse serum were given, over 90 per cent of the patients would develop serum disease, whereas if 10 c.c. or less were administered, only 10 per cent would be so afflicted.[3] More recently, Moynihan[7] followed 7,580 patients who received purified prophylactic antitoxin (usually 1.0 c.c. of antitetanus de-speciated horse serum globulin). He reported that only 2.5 per cent developed serum sickness. Also, Sansöe-Jensen and Zachau-Christiansen[8] noted that sixty-four of 1,273 patients who received prophylactic tetanus antitoxin had adverse reactions from this foreign protein, but only twenty had serum sickness. Hence, the incidence in this series was only 1.6 per cent. It must be assumed, therefore, that the incidence of serum sickness has been markedly reduced due to further purification of the horse serum and the injection of smaller volumes of tetanus antitoxin.

It has been shown that patients who develop serum sickness following injections of horse serum have demonstrable antibodies, such as precipitins[9, 10] and anaphylactic and skin-sensitizing antibodies. All these earlier studies were done with sera from patients who received large amounts of relatively unpurified horse serum. However, several investigators have been unable to demonstrate precipitins in patients with serum sickness.[11, 12]

It seemed rather strange to us that all or a great percentage of patients would not develop antibodies against as potent an antigen as horse serum. Could this be due to the fact that our methods of determining antibody were

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too insensitive? In recent years a more delicate and sensitive technique of determining antibodies of the precipitin type (the indirect hemagglutination technique) has been developed.[13-15] We used this method to determine the effect of injections of prophylactic purified tetanus antitoxin on the hemagglutinin titer and to compare our findings with the antibodies determined by the usual precipitin methods and the passive transfer technique.

The antigenic relationship between horse serum and dander and between other animal danders and sera[16-18] has been recognized for many years. By the use of the hemagglutination methods, we have shown certain interesting cross-relationships.[19]

The length of time that the precipitin type of antibody persists after serum sickness, as determined by the classical methods, has been reported to vary considerably. Many workers in this field have mentioned the difficulties and inadequacies of the precipitin test.[20, 21] In order to overcome some of these obstacles, we employed the hemagglutination method to determine the length of time that antibodies remain in the circulation following injections of prophylactic "purified" tetanus antitoxin. It has been shown that this method of measuring antibodies of the "precipitin type" is approximately 1,000 times as sensitive as the precipitin test.[22] We hoped that, by using this technique, we could detect the presence of minute amounts of circulating antibodies against horse serum.

METHODS AND MATERIALS

Source of Sera.—Patients admitted to the emergency room of the Buffalo General Hospital who required prophylactic injections of tetanus antitoxin were studied. Only those patients who gave a negative intradermal skin test to horse serum were injected with the tetanus antitoxin. Blood samples were taken, with sterile precautions, before the administration of the tetanus antitoxin. The patients were instructed to return in two to three weeks following the injection for another blood test. If symptoms of serum disease developed prior to this time, they were advised to return immediately and serum was collected. Several patients with serum disease had follow-up samples collected over a period of many months, 472 days being the longest period.

Sera from patients who developed serum sickness but from whom we had no pretreatment samples were also studied. Sera from patients clinically sensitive to horse and other animal danders, ragweed pollen, and other substances were likewise employed in this investigation.

All of these sera were kept frozen until ready for experimental analysis.

Rabbits were immunized with "purified" horse serum globulin by daily injections of 1.0 c.c. subcutaneously for three days. The animals were bled out seven to ten days following the last injection. A second series of rabbits was injected with horse dander extract mixed with an equal volume of complete Freund adjuvant (1.0 c.c. each), and the rabbits were sensitized in the same manner as with the horse serum. These sera served as positive controls.

Hemagglutination Technique.—The details of this technique are described in another publication.[23]

1. 0.5 ml. of the antisera was prepared in serial dilutions in normal rabbit serum 1:100, as were controls of normal human sera and normal rabbit sera.

2. Thrice washed in pH 7.2 buffered saline,* citrated human group O, Rh-negative red blood cells were resuspended to 2.5 per cent in pH 7.2 buffered saline and treated with equal parts of tannic acid† 1:20,000 for ten minutes at 37° C. After centrifugation for five minutes at 1,200 r.p.m., the red blood cells were washed in the same volume of pH 7.2 buffered saline, and the tanned red blood cells were then resuspended in normal saline to 2.5 per cent. The cells were "sensitized" by adding one part of red blood cells to four parts of pH 6.4 buffered saline‡ and one part of antigen (horse serum diluted 1:200 with buffered saline). This mixture was then incubated for fifteen minutes at room temperature and centrifuged. After one washing with two volumes of 1:100 normal rabbit serum, the tanned sensitized red blood cells were made up as a 2.5 per cent suspension in normal rabbit serum 1:100, and 0.05 c.c. was added to serial dilutions of antisera.

3. Controls consisted of normal rabbit serum, normal human serum with tanned sensitized red blood cells, and test sera with tanned but unsensitized red blood cells.

4. The tubes were shaken, and the hemagglutination patterns were recorded after two hours at room temperature and again after they had remained in an icebox overnight.

5. The hemagglutination patterns remained stable, without lysis, for as long as twenty-four hours if kept at room temperature and for three to four days if kept in the icebox. There was no apparent elution of antigen.

The same method was applied when other antigens, such as tetanus antitoxin (purified de-speciated horse serum globulin) from two different commercial sources, and other mammalian sera were used to coat the tanned red blood cells.

Bis-diazotized benzidine method: When horse dander was employed as the antigen, it was necessary to use the bis-diazotized benzidine (B.D.B.) method

*pH 7.2 buffered saline, 0.15 M solution:
8.15 Gm. Na_2HPO_4
2.45 Gm. KH_2PO_4
4.5 Gm. NaCl
1,000 c.c. distilled H_2O

†*Normal saline:* The normal saline used was 0.85 per cent of NaCl solution.

Tannic acid: A 1:200 solution of tannic acid crystal ($C_{14}H_{10}O_9$) (not fluffy) in H_2O was kept in stock for as long as ten days and diluted 1:20,000 in normal saline for the experiment.

‡pH 6.4 buffered saline, 0.15 M solution:
3.44 Gm. Na_2HPO_4
6.92 Gm. KH_2PO_4
4.5 Gm. NaCl
1,000 c.c. distilled H_2O

of hemagglutination as previously described.[15, 19] Horse dander in a 1:10 dilution of an extract (phosphotungstic acid nitrogen, 1.96) was used as the antigen to be linked to the red blood cells.

Inhibition experiments (horse dander and horse serum): Varying dilutions of horse dander extract were mixed with a constant amount of a patient's serum in a dilution known to give a good positive hemagglutination reaction. These mixtures were allowed to stand at room temperature for three hours. Then the tanned red blood cells coated with horse serum were added to each of these mixtures. The patterns were read in two hours and after being in the icebox overnight. The reverse experiments were used, inhibiting the reaction with horse serum and linking the red blood cells to the horse dander by the B.D.B. method.

Precipitin Determinations.—To various dilutions of horse serum (0.1 c.c. diluted in normal saline) a constant amount of concentrated antisera (0.1 c.c.) was added. Ring formations were read, and then the tubes were shaken and kept at room temperature for three hours and overnight. The flocculation was then recorded with the aid of the agglutinoscope.

Passive Transfer (Reagin) Determinations.—The posttreatment sera of patients who developed serum sickness were studied for passive transfer antibodies. These sera were first tested for sterility, and then 0.1 c.c. of the serum was injected intradermally into each of two recipients who were skin-test negative to horse serum. The sites were marked, and twenty-four hours later each site was tested with a 1:10 dilution (0.1 c.c.) of horse serum. The reactions were read in twenty minutes.

RESULTS

There was a total of 122 patients from whom sera were obtained for study. From this group we obtained pretreatment samples (prior to the injection of tetanus antitoxin) from seventy-eight patients. Unfortunately, only forty-two patients returned for posttreatment studies. However, we did obtain additional posttreatment sera from thirty-seven patients from whom no pretreatment sera was available. Hence, the effect of an injection of prophylactic tetanus antitoxin on the hemagglutination titer of sera from seventy-nine patients was determined.

The hemagglutination titers of the seventy-eight pretreatment patients are summarized in Table I. Only three of this entire group had titers over 2,000.

TABLE I. HEMAGGLUTINATION TITERS AGAINST HORSE SERUM IN SEVENTY-EIGHT PATIENTS PRIOR TO A PROPHYLACTIC TETANUS ANTITOXIN INJECTION

TITER RANGE*	NUMBER OF PATIENTS
0	41
10-20	15
40-200	8
>200-2,000	11
6,250	1
32,000	1
160,000	1
Total	78

*Reciprocal of highest dilution of antiserum which still gave a positive hemagglutination reaction.

The hemagglutination titers of the posttreatment sera varied from 0 to 5,000,000. The range of titers is illustrated in Table II. Thirty-three patients had no antibody demonstrable by this sensitive technique, despite a previous injection of tetanus antitoxin. An additional nineteen patients had titers of 2,000 or less.

TABLE II. HEMAGGLUTINATION TITERS FOLLOWING A PROPHYLACTIC INJECTION OF TETANUS ANTITOXIN

TITER RANGE	NUMBER OF PATIENTS
0	33
10-20	7
40-200	6
200-2,000	6
2,000-8,000	4
8,000-30,000	2
30,000-100,000	6
100,000-400,000	8
400,000	7
Total	79

Of the 42 patients with pre- and post-treatment sera the titers showed an increase in eighteen and remained unchanged in twenty-two (Table III). Of the eighteen patients who showed an increase in titer following the tetanus antitoxin injection, four had a fourfold increase, three patients had an eightfold increase, and eleven developed an increase of sixteen fold or greater.

TABLE III. HEMAGGLUTINATION TITERS PRIOR TO AND AFTER INJECTION OF TETANUS ANTITOXIN

TITER CHANGE	NUMBER OF PATIENTS	
None	22	
Increase		
Four fold	4	18
Eight fold	3	
Sixteen fold or greater	11	
Decrease	2	

Nine of the forty-two patients developed serum sickness. Seven of these nine patients had an increase in titer of sixteen fold or greater and, furthermore, all but one of these nine patients had an initial (pretreatment) titer of 1,000 or more. The patients who did not develop serum sickness all had pretreatment titers of 200 or less. Twenty-two of these patients had a negative titer, but six developed positive titers following the tetanus antitoxin injection. Only one of these patients (without serum disease) produced a titer above 2,000 (Fig. 1).

Relationship of Hemagglutination Titers to Serum Sickness.—In addition to the nine patients of this group of forty-two who developed serum disease, another fifteen of the thirty-seven patients from whom we were able to obtain sera only following tetanus antitoxin injection also had serum disease. The post-treatment titers of these seventy-seven are listed in Table IV. All patients with serum sickness had titers of more than 2,000. The lowest titer was 4,000; this was from the serum of a patient who had recovered from serum sickness 100 days previously. The length of time from the injection of tetanus antitoxin to the obtaining of the blood sample varied from eight days to fifteen months.

Hence, in summarizing these various groups, twenty-four patients had serum sickness and all had titers greater than 2,000, whereas fifty-three had no ill effects from the tetanus antitoxin and only one of these had a titer greater than 2,000.

Studies of Sera From Patients Who Had Serum Sickness.—The hemagglutination titers of the twenty-four patients with serum sickness are listed in Table V. Also listed are the titers obtained when the tests were performed with tetanus antitoxin from two different commercial sources, Companies X and Y, as the antigens. In each case the optimal dilution of antigen for continued red blood cells was determined in preliminary experiments. It will

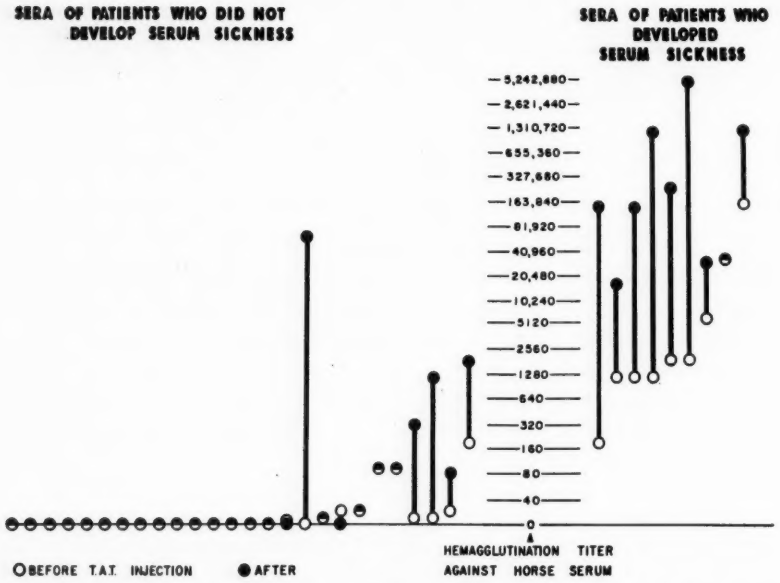

Fig. 1.—Comparative hemagglutination titers against horse serum of sera from patients prior to and following the injection of prophylactic tetanus antitoxin. Only those patients with an *original* titer of 160 or greater developed serum sickness. Just one patient whose titer, after tetanus antitoxin, was above 2,000 did not have serum sickness. Of the nine patients who developed serum sickness, seven denied receiving any previous horse serum therapy at any time in their lives. However, one patient had an injection of tetanus antitoxin in 1940 without reaction, and another patient had a similar serum sickness reaction in 1939.

TABLE IV. HEMAGGLUTINATION TITERS FOLLOWING TETANUS ANTITOXIN INJECTIONS

TITER RANGE	PATIENTS WITH SERUM SICKNESS	PATIENTS WITHOUT SERUM SICKNESS
0		31
10-20		7
40-200		5
200-2,000		9
2,000-8,000	2	
8,000-30,000	2	
30,000-100,000	5	1
100,000-400,000	7	
Greater than 400,000	8	
	Total 24	53
	Total 77	

TABLE V. ANTIBODY TITERS OF SERA FROM PATIENTS WITH PREVIOUS SERUM DISEASE

HUMAN ANTISERA	HEMAGGLUTINATION* ANTIGENS: HORSE SERUM	TETANUS ANTITOXIN: X	TETANUS ANTITOXIN: Y	DANDER†	PRECIPITATION‡
1. P. A.		3,200	200	1,280	0
2. A. B.	2,560,000	640,000	6,400	80	2,000
3. J. B.	31,250	12,800		640	0
4. C. U.	8,000	400		320	2,000
5. M. C.	16,000	3,200		320	0
6. R. D.	128,000	80,000		160	100
7. M. E.	320,000	20,000		640	0
8. G. J.	640,000	160,000		1,280	2,000
9. J. H.	1,512,000	160,000		5,120	
10. J. J.	528,125	25,600	2,400	160	100
11. W. L.	128,000	640,000		2,560	2,000
12. J. M.	128,000	12,800	320	2,560	2,000
13. M. M.	128,000	400		160	0
14. H. M.	64,000				0
15. A. M.	16,000	12,800		60	0
16. M. Mc.	32,000	1,600		320	0
17. M. P.	4,000	600		320	0
18. H. S.	1,000,000	51,200	0	1,280	10
19. V. S.	128,000				2,000
20. E. S.	1,024,000	60,000		10,240	100
21. G. S.	1,280,000	80,000		640	0
22. R. S.	128,000	0		640	10
23. Turr.	80,000	10,000		5,120	
24. A. W.	5,120,000	500	2,000	2,560	1,000

*Reciprocal of highest dilution of human antiserum which gave a positive hemagglutination with tanned red blood cells coated with horse serum, on tetanus antitoxin from Companies X and Y.

†Reciprocal of highest dilution of human antiserum which gave positive hemagglutination when red blood cells were linked by bis-diazotized benzidine to horse dander.

‡Reciprocal of highest dilution of horse serum (antigen) which gave a positive precipitin test with undiluted antisera (antibody).

be noted that the titer was definitely decreased when these antigens were used for the hemagglutination tests. The weakest reaction by this technique was obtained when de-speciated tetanus antitoxin horse globulin from Company Y was used. When horse dander was used as the antigen, titers were considerably lower than when the natural horse serum was employed, but in each instance positive reactions were obtained. The precipitin titers were determined in most of these patients. Despite the high hemagglutination titers, eleven sera failed to show any precipitins and the strongest reaction occurred in a 1:2,000 dilution of antigen.

The presence of skin-sensitizing antibody was determined in twenty-two of these twenty-four sera. Negative tests were found in eight and definite positives were found in the remaining fourteen sera. It was noted that the sera from patients with a longer interval between the serum disease and the bleeding had lower amounts of passive transfer antibodies than those patients whose serum was obtained within two months following the illness. This was not always the case, however. One patient who had serum sickness 460 days previously still showed a positive reagin reaction.

Follow-up of sera: The hemagglutination titers of patients with initially high titers after tetanus antitoxin injection were followed intermittently for

many months. Table VI illustrates the gradually decreasing titers. Four of these patients had blood samples taken prior to the injection of tetanus antitoxin. The high titers were demonstrated within twenty-four to forty-eight hours after the onset of a serum sickness, and high but decreasing titers have lasted as long as 472 days. We obtained serum from one patient who developed serum sickness sixteen years ago. Her titer was still 16,000.

TABLE VI. FOLLOW-UP HEMAGGLUTINATION TITERS OF SERA FROM PATIENTS WHO HAD SERUM SICKNESS

Patient A. W.	Days post-treatment	10	25	68	149	160	294	472
	Titer	5,120,000	2,560,000	320,000	160,000	128,000	64,000	64,000
Patient H. Sch. Pretreatment 1,000	Days post-treatment	9	15	41	71	138	232	
	Titer	1,000,000	2,000,000	160,000	80,000	31,250	8,000	
Patient J. M. Pretreatment 2,000	Days post-treatment	8	18	52	116	334		
	Titer	100,000	2,000,000	512,000	80,000	4,000		
Patient B. M.	Days post-treatment	198	460					
	Titer	128,000	10,000					
Patient A. B. Pretreatment 1,000	Days post-treatment	10	44	279				
	Titer	781,000	320,000	2,000				
Patient W. L. Pretreatment 160,000	Days post-treatment	7	21	93				
	Titer	1,280,000	2,560,000	1,200				

Inhibition tests: A constant amount of a dilution of human serum (known to contain hemagglutinating antibodies against horse serum) was added to horse serum in varying dilutions and then incubated at room temperature for two hours. Red blood cells coated with horse serum were then added to these mixtures. The patterns were read in the usual manner. The minimal amount of native horse serum necessary to inhibit the specific hemagglutination reaction could then be determined. Table VII represents such an experiment, and it can be seen that as little as a 1:512,000 dilution of horse serum can prevent the reaction of a 1:16,000 dilution of the human serum (containing hemagglutinating antibodies) and the red blood cells coated with horse serum. Also worthy of note is the linear relationship that exists—a twofold dilution of antibody required approximately one-half the amount of antigen (horse serum) to inhibit the reaction.

A comparison of the inhibition properties of two commercial tetanus antitoxin preparations and native horse serum is illustrated in Table VIII. Only one part in approximately 312,500 of native horse serum was necessary to inhibit the hemagglutination reaction when the antibody was diluted 1:30,000. However, a dilution of 1:62,500 of tetanus antitoxin X was necessary to inhibit the same dilution of antibody, and a 1:2,500 dilution of tetanus Y was required

TABLE VII. INHIBITION OF HEMAGGLUTINATION OF HUMAN ANTIHORSE SERUM AND TANNED RED BLOOD CELLS COATED WITH HORSE SERUM BY NATIVE HORSE SERUM

0.5 C.C. OF DILUTIONS OF HORSE SERUM IN 1% NRS INHIBITING ANTIGEN	ANTIHORSE HUMAN SERUM (0.1 C.C.) DILUTIONS*					
	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000	1:32,000
1:16,000	0	0	0	0	0	0
1:32,000	0	0	0	0	0	0
1:64,000	+	0	0	0	0	0
1:128,000	+	+	0	0	0	0
1:256,000	+	+	0	0	0	0
1:512,000	+++	+	+	0	0	0
1:1,024,000	+++	+	+	+	+	0
1:12,048,000	+++	+	++	++	++	+
1:500	0	0	0	0	0	0

+ = Uncoated red blood cells.

*The mixtures of the inhibiting antigen dilutions and the antibody (constant dilutions of antihorse human serum) were kept at room temperature for two hours prior to the addition of tanned red blood cells coated with horse serum.

TABLE VIII. COMPARATIVE INHIBITION OF HEMAGGLUTINATION OF HUMAN ANTIHORSE SERUM AND TANNED RED BLOOD CELLS COATED WITH HORSE SERUM BY HORSE SERUM AND TWO COMMERCIAL TETANUS ANTITOXIN PREPARATIONS

0.5 C.C. OF DILUTIONS OF INHIBITING ANTIGEN DILUTED IN NRS 1:100	ANTIHORSE HUMAN SERUM 0.1 C.C.* DILUTED 1:30,000			
	TETANUS ANTITOXIN X	TETANUS ANTITOXIN Y	HORSE SERUM	NORMAL RABBIT SERUM (CONTROL)
1:2,500	0	0	0	+++
1:12,500	0	++	0	+++
1:62,500	0	++	0	+++
1:312,500	+	++	0	+++
1:1,562,500	+++	++	+	+++
1:7,812,500	+++	+++	+	+++
1:39,062,500	+++	+++	++	+++
1:195,312,500	+++	+++	++	+++
1:966,562,500	+++	+++	++	+++
1:100	0	0	0	0

+ = Uncoated red blood cells.

*The mixtures of the inhibiting antigen dilutions and the antibody (constant dilutions of antihorse human serum) were kept at room temperature for two hours prior to the addition of tanned red blood cells coated with horse serum.

to produce the same result. These data conform quite well with the results of direct hemagglutination when coating the tanned red blood cells to the different tetanus antitoxins and horse serum. Native horse serum as the coating antigen gave higher titers than either tetanus antitoxin preparation, but preparation X gave higher titers than preparation Y.

The sera of twenty-one patients who had negative hemagglutination titers following a prophylactic tetanus antitoxin injection were used as inhibiting agents in the same manner that native horse serum and the tetanus antitoxin preparations were used in the previous experiments. This was done in order to determine whether free antigen (horse serum) was still present in the circulation of these patients. The only difference in technique was that lower dilutions of the human sera (starting with a 1:10 dilution) were used. An example of this type of inhibition test is shown in Table IX. As can be seen,

one serum (L.), exhibited no inhibition, but sera M. and A. prevented the hemagglutination reaction in dilutions of 1:80 and 1:40, respectively. However, only a 1:6,400,000 dilution of tetanus antitoxin X and a 1:800,000 dilution of tetanus antitoxin Y were required to cause this same inhibition.

TABLE IX. COMPARATIVE INHIBITION OF HEMAGGLUTINATION OF HUMAN ANTIHORSE SERUM AND TANNED RED BLOOD CELLS COATED WITH HORSE SERUM BY SERA FROM PATIENTS FOLLOWING TETANUS ANTITOXIN INJECTION

0.5 c.c. of dilutions of inhibiting sera in 1 % NRS	Antihorse human serum 0.1 c.c.* diluted 1:10,000				0.5 c.c. of dilutions of tetanus antitoxin in 1 % NRS	Tetanus antitoxin	
	Name: Days post-treatment	Serum L. 14	Serum M. 6	Serum A. 8		X	Y
1:10		++	0	0	1:800,000	0	0
1:20		++	0	0	1:1,600,000	0	++
1:40		++	0	0	1:3,200,000	0	+
1:80		+	0	+	1:6,400,000	0	+
1:160		+	+	+	1:12,800,000	+	+
1:320		+	+	+	1:2,560,000	+	+
Control		0	0	0		0	0

*The mixtures of the inhibiting antigen dilutions and the antibody (constant dilutions of antihorse human serum) were kept at room temperature for two hours prior to the addition of tanned red blood cells coated with horse serum.

Of the twenty-one sera tested, nine failed to produce any inhibition, but circulating antigen (tetanus antitoxin) was present in the remaining twelve specimens, as evidenced by inhibition in titers as high as 1,280 as long as sixteen days following a prophylactic injection of tetanus antitoxin.

Inhibition of the hemagglutination reaction between horse serum coated on tanned red blood cells and human antihorse serum by horse dander extract is illustrated in Table X. It will be noted that it took a 1:40 dilution of a 10

TABLE X. INHIBITION OF HEMAGGLUTINATION BY HORSE DANDER OF HUMAN ANTIHORSE SERUM AND TANNED RED BLOOD CELLS COATED WITH HORSE SERUM

Dilutions of horse dander in NRS 1:100 0.5 c.c. inhibiting antigen	0.1 c.c. J.M. human antihorse serum dilutions*	
	1:1,000	1:10,000
1:20	0	0
1:40	0	0
1:80	+	0
1:160	+	0
1:320	+	0
1:640	+	0
1:1,280	+	0
1:2,560	+	0
1:5,120	+	+
NRS	++	++
1:20	0	0

+ = Uncoated red blood cells.

*The mixtures of the inhibiting antigen dilutions and the antibody (constant dilutions of antihorse human serum) were kept at room temperature for two hours prior to the addition of tanned red blood cells coated with horse serum.

per cent saline extract of horse dander to inhibit a 1:1,000 dilution of human antihorse serum, whereas only a 1:32,000 dilution of native horse serum produced the same effect (Table VII).

Contrariwise, when native horse serum was compared to horse dander in the inhibition of a rabbit antihorse dander serum and horse dander linked by bis-diazotized benzidine to the red blood cells, a 1:24 dilution of horse serum and a 1:320,000 dilution of horse dander extract were required to produce the same effect (Table XI).

TABLE XI. INHIBITION OF HEMAGGLUTINATION OF RED BLOOD CELLS LINKED TO HORSE DANDER (BY B.D.B.) AND RABBIT ANTIHORSE DANDER SERUM BY HORSE DANDER AND HORSE SERUM

	0.1 C.C. PER TUBE OF RABBIT ANTI-HORSE DANDER SERUM (R. c.-20)* 1:10,000 DILUTIONS OF ANTIGENS 0.5 C.C. IN 1% NRS				
DILUTIONS OF HORSE DANDER IN NRS 1:100 0.5 C.C. PER TUBE	HORSE DANDER	HORSE SERUM		NRS	NRS WITHOUT RABBIT ANTI-SERUM
1:10	–	1:3	–	++	–
1:100	–	1:6	–	++	–
1:1,000	–	1:12	–	++	–
1:10,000	–	1:24	–	++	–
1:20,000	–	1:48	+	++	–
1:40,000	–	1:96	++	++	–
1:80,000	–	1:192	++	++	–
1:160,000	–	1:384	++	++	–
1:320,000	–	1:768	++	++	–
1:640,000	+	1:1,532	++	++	–
1:1,280,000	++	1:3,064	++	++	–
1:10 plus uncoated red blood cells	–	1:3	–	–	–

*The mixtures of the inhibiting antigen dilutions and the antibody (constant dilutions of the antihorse human serum) were kept at room temperature for two hours prior to the addition of red blood cells linked to horse dander by B.D.B.

Relationship of other mammalian sera to horse serum: Hemagglutination tests were made, using the serum of a patient who had serum sickness and coating the tanned red blood cells with various mammalian sera. It can be seen (Table XII) that all except rat sera gave some reaction, but in a much lower titer than did the horse serum.

TABLE XII. HEMAGGLUTINATION OF SERUM FROM PATIENTS WITH SERUM SICKNESS AND VARIOUS MAMMALIAN SERA AS ANTIGENS

SERUM	TITER
Mouse	800
Guinea pig	800
Cat	500
Rabbit	1,600
Hog	2,000
Lamb	200
Rat	0
Horse	512,000

DISCUSSION

Although the incidence of serum disease following a prophylactic injection of the newer preparations of tetanus antitoxin is markedly decreased, due probably to smaller volume and more purified material, serum sickness is still frequently encountered.

It is often difficult to determine whether the signs and symptoms are due to the administration of the serum or to some other medication, such as penicillin. Both of these substances are frequently given at the same time following an injury. A test of the serum for hemagglutinating antibodies against horse serum could differentiate between these two possibilities. Of course, a simple precipitin test might be performed, but, as we have shown, if the titer is not sufficiently elevated, this test may be negative. The hemagglutination test is much more sensitive and easier to interpret. As a matter of fact, in those patients whose sera contain very high titers, when the coated and tanned red blood cells are added to the more concentrated dilutions, hemagglutination and clumping appear within five minutes.

Recenty Zimmerman[25] proposed that a method of differentiating between a penicillin reaction and a horse serum reaction should be the administration of penicillinase to the patient. If the patient's symptoms cleared up, it could be assumed that the reaction was due to penicillin. It has been our personal experience, and also that of the Committee on Drugs of the American Academy of Allergy,[26] that penicillinase is certainly not completely effective (less than 50 per cent) in this type of penicillin reaction.

In the group of forty-two patients from whom we obtained sera prior to the tetanus antitoxin injection, it is interesting to note that of the nine who subsequently developed serum sickness all but one had titers of 1,000 or greater, whereas of the remaining thirty-three patients all but one had a titer below 200. This fact, too, could perhaps be an index of those patients who could conceivably develop serum disease. It is known that a negative intradermal test does not mean that the patient will avoid having serum disease. As a matter of record, all of our forty-two patients had negative intradermal skin tests against the tetanus antitoxin; yet nine of them developed serum sickness. Hence, in addition to an intradermal test prior to the administration of horse serum in order to avoid the atopic or accelerated type of serum reaction, a hemagglutination test might well be performed. If a titer of 1,000 or greater was obtained prior to the tetanus antitoxin injection, one could anticipate that the patient would develop primary serum sickness. The patient could be forewarned or carefully observed, and perhaps he could be given antihistaminics and/or corticosteroids in an attempt to prevent this reaction.

Although it is well known that both the antihistaminics and the corticosteroids will alleviate the symptoms of serum disease, once they have developed, their value as prophylactic agents for serum sickness have not as yet been proved in human patients. However, several investigators have demonstrated that the corticosteroids will prevent lesions produced in animals by horse serum if these steroids are given prior to administration of the antigen (horse serum). This, then, could lead us to another set of experiments in which one-third of all patients with pretreatment titers of 1,000 or greater against horse serum would be given corticosteroids, one-third would be given antihistaminics, and one-third would serve as a control group.

Our inhibition experiments have shown that it takes a very minute amount of antigen (either native horse serum or a commercially available tetanus antitoxin) to prevent hemagglutination. Perhaps the reason that we obtained negative or relatively low titers in all patients except those who had serum sickness was that there was enough circulating antigen (tetanus antitoxin) present in the serum to inhibit the reaction. MacKenzie and Hanger[3] have shown that the antigen can be detected in the serum for as long as sixty-seven days in those patients who do not develop serum sickness. Because of the sensitivity of this inhibition test, circulating antigen in very minute amounts can be determined. This was indeed the case. Titers of sera from patients who had negative hemagglutination reactions following tetanus antitoxin injection inhibited a known antigen antibody system up to a dilution of 1:1,280. This compared to a 1:6,000,000 dilution of tetanus antitoxin. If one were to consider that the 0.5 c.c. of prophylactic tetanus antitoxin is diluted by 3.5 liters of plasma, then the initial dilution of the antigen would be approximately 1:7,000—enough to show circulating antigen.

Unfortunately, it was not possible to carry out this type of inhibition test with sera of those patients who had horse serum antibodies. The direct agglutination by the antibodies masked any inhibition.

The hemagglutinating antibody appears shortly after the development of the symptoms of serum sickness and may last in elevated but diminishing titers for at least fifteen months. We studied the serum of one patient who had serum disease fifteen years ago and still had a relatively high titer. The other antibodies found in serum disease—the precipitins, anaphylactic antibodies, and reagins—disappeared long before the hemagglutinins. These may be different antibodies. In 1947 Loveless[27] showed that the precipitins could be removed from the serum and the reagin still be demonstrated.

Several authors[4, 12] found no relationship between precipitins and serum sickness. Our findings would indicate, however, that almost all patients with a hemagglutination titer over 2,000 either had or have serum disease. Whether this elevated titer is the cause or the result of the serum sickness cannot as yet be determined. We uniformly obtained lower hemagglutination titers when the commercial preparations of tetanus antitoxin were used to coat the tanned red blood cells. These findings could be due to the fact that, in the preparation of the purified horse serum globulin of tetanus antitoxin, a product is developed which does not readily or completely attach to the red cell. Also, certain of the protein components, which could conceivably be antigenic, are either altered or are present in such small quantities that they could not be detected. One of the two commercial tetanus antitoxin products appeared much "weaker" for these tests than the other. Unfortunately, we have no data as to which of these products was injected into our patients and the relative incidence of serum sickness produced.

We also reported that it required more tetanus antitoxin than native horse serum to inhibit a hemagglutination reaction.

The antigenic relationship between horse dander and horse serum has been carefully worked out by several investigators. Stanworth[16] and Squire[17] have demonstrated that the serologic relationship is due to the presence of small quantities of serum albumin and globulin in the dandruff material. Silver and Bookman[18] likewise showed a common albumin fraction between dander and serum. In our studies of direct hemagglutination and inhibition tests, we found that horse dander extract can give positive but lower titers than the horse serum. We were indeed surprised that there was such a lack of common antigenicity between horse dander and horse serum as evidenced by our experiments. It can be recalled that it required a 1:24 dilution of horse serum to produce the same degree of inhibition as a 1:320,000 dilution of 10 per cent extract of horse dander for an antihorse rabbit serum. Likewise, a 1:40 dilution of horse dander was necessary to inhibit, as compared to a 1:32,000 of horse serum for an antihorse-serum human serum. Moreover, several of our horse-dander-sensitive patients did not show titers against horse serum in the hemagglutination test.

In a previous communication,[19] we had shown not only the relationship of various animal danders to sera but also the cross-reactivity between various mammalian sera. In our present report, it has been demonstrated that a patient with a high hemagglutination titer against horse serum also had some reaction to other mammalian sera. This may be of practical significance, as patients sensitive to horse serum are often given bovine serum as a substitute. In view of our findings, one should use the same precautions (namely, intradermal skin tests) with this second serum before administering another serum. However, a negative skin test for either serum does not necessarily preclude the patient's developing serum sickness. All of our patients with pre- and posttreatment sera were previously skin tested, and still nine of these forty-two developed classical serum sickness. However, Kojis[4] showed that the incidence of serum sickness was greater in patients who had a positive skin test.

There are several antigens in tetanus antitoxin and, consequently, perhaps several antibodies develop. We do not know which fraction or fractions of the horse serum protein produce serum sickness or, for that matter, what portion of the antigen coats on the red blood cell and demonstrates the hemagglutinating antibody. Studies are now underway in an attempt to separate the horse serum antigen and also the fraction of the human serum which produces this antigen-antibody reaction.

SUMMARY AND CONCLUSIONS

1. The tanned-cell hemagglutination test for horse serum antibodies is specific, reproducible, and extremely sensitive. Patients who developed serum sickness showed high titers (above 2,000).

2. Only those patients who had a hemagglutination titer of 160 or greater *prior* to the injection of tetanus antitoxin developed serum sickness.

3. This technique may prove useful in the differentiation between "serum sickness syndrome" produced by horse serum and that produced by penicillin.

4. There is only slight cross-reactivity between horse dander and horse serum, but there is greater common antigenicity between various mammalian sera and horse serum.

5. There is a smaller but significant rise in hemagglutinating antibody titer (to under 2,000) in many of the patients who receive tetanus but do not develop serum sickness.

We wish to thank Mrs. Hazel Popp and June Spohr for their technical assistance and Dr. Elias Cohen of the Roswell Park Memorial Institute for supplying us with the antihorse serum and antihorse-dander rabbit sera. We are also grateful to Mrs. W. Stafford, Jr., for her financial support to our laboratory.

REFERENCES

1. Von Pirquet, C. F., and Schick, B.: Die Serum Krankheit, Leipzig-Wien, 1905 (English translation by Schick, B.: Serum Sickness, Baltimore, 1951, Williams & Wilkins Company).
2. Waever, G. H.: Serum Disease, Arch. Int. Med. **3**: 483, 1909.
3. MacKenzie, G. M., and Hanger, F. M.: Serum Disease and Serum Accidents, J. A. M. A. **94**: 260, 1930.
4. Kojis, F. G.: Serum Sickness and Anaphylaxis, Am. J. Dis. Child. **64**: 93, 313, 1942.
5. Longcope, W. T.: Serum Sickness and Analogous Reactions From Certain Drugs, Particularly the Sulphonamides, Medicine **22**: 251, 1943.
6. Ratner, B.: Allergy, Anaphylaxis and Immunology, Baltimore, 1943, Williams & Wilkins Company.
7. Moynihan, N. H.: Serum Sickness and Local Reactions in Tetanus Prophylaxis; a Study of 400 Cases, Lancet **2**: 264, 1955.
8. Sansöe-Jensen and Zachau-Christiansen: Ugesk. Laeger, Sept. 11, 1958 (cited in J. A. M. A. **168**: 1923, 1958).
9. Hamburger, F., and Moro, E.: Über die Biologisch Nachweisbaren Veraenderungen des Menschlichen Blutes nach der Serum Injektion, Wien. Klin. Wchnschr. **16**: 445, 1903.
10. Wells, C. W.: On the Specific Precipitin in the Blood of Persons Injected With Antidiphtheria Horse Serum, J. Infect. Dis. **16**: 63, 1915.
11. Wyard, S.: Precipitins and the Etiology of Serum Sickness, J. Path & Bact. **25**: 191, 1922.
12. Tuft, L., and Ramsdell, S. G.: The Antibody Response in the Human Being After Injection With Horse Serum, J. Exper. Med. **50**: 431, 1929.
13. Boyden, S. V.: The Adsorbtion of Protein on Erythrocytes Treated With Tannic Acid and Subsequent Hemagglutination by Anti-Protein Sera, J. Exper. Med. **93**: 107, 1951.
14. Stavitsky, A. B.: Micromethods for the Study of Protein and Antibodies. Parts I and II, J. Immunol. **72**: 360-367, 368-375, 1954.
15. Gordon, J., Rose, B., and Sehon, A. H.: Detection of "Non-Precipitating" Antibodies in Sera of Individuals Allergic to Ragweed by an In Vitro Method, J. Exper. Med. **108**: 37, 1958.
16. Stanworth, D. R.: The Use of the Gel Precipitation Technique in the Identification of Horse Dandruff Allergen, and in the Study of the Serological Relationship Between Horse Dandruff and Horse Serum Protein, Internat. Arch. Allergy **2**: 170, 1957.
17. Squire, J. R.: The Relationship Between Horse Dandruff and Horse Serum Antigens in Asthma, Clin. Sc. **9**: 127, 1950.
18. Silver, D. Z., and Bookman, R.: Studies on the Nature of Allergenic Material in Animal Danders and Pollens, J. ALLERGY **27**: 127, 1956.
19. Beede, R. B., Rose, N. R., and Arbesman, C. E.: Sensitivity to Animals; Case Report With Immunologic Studies. II. Serologic Aspects, J. ALLERGY **29**: 139, 1958.
20. Cannon, P. R., and Marshall, C. E.: Studies on the Mechanism of the Arthus Phenomenon, J. Immunol. **40**: 127, 1941.
21. Tuft, L., and Ramsdell, S. G.: Antibody Studies in Serum Sickness, J. Immunol. **17**: 539, 1929.
22. Grabar, P.: Comparison of Quantitative Immunological Methods, Abst. Comm. VI International Congress of Microbiology, Rome, 1953, p. 1477.
23. Kantor, S. Zwi, and Arbesman, C. E.: Serologic Studies on Favism, J. ALLERGY **30**: 114, 1959.

24. Arbesman, C. E., Hyman, I., Dauzier, G., and Kantor, S. Zwi: Immunologic Studies of Guillian-Barré Syndrome Following Tetanus Antitoxin, New York J. Med. 58: 2647, 1958.
25. Zimmerman, M. C.: The Prophylaxis and Treatment of Penicillin Reactions With Penicillinase, Clin. Med. 5: 305, 1958.
26. Friedlaender, S.: Penicillinase in the Treatment of Allergic Reactions to Penicillin, J. ALLERGY 30: 181, 1959.
27. Loveless, M. H.: Immunological Studies on Serum Disease. I. Fluctuations in the Precipitin Nitrogen During Four Months Following a Serum Reaction, Fed. Proc. 6: 429, 1947.

AMERICAN ACADEMY OF ALLERGY

RESEARCH IN THE FIELD OF ALLERGY

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SOME months ago the widow of Dr. William Schultz consulted me about her desire to establish a memorial to her husband. I suggested that she endow a lectureship with the American Academy of Allergy. The idea was acceptable to the officers of the Academy and to Mrs. Schultz. Little did I think at the time that the honor as well as the task of preparing the first lecture would fall to me.

I have read reprints of most of Dr. Schultz's published work. They are impressive, and in them one can see that William Schultz was both an exceptional teacher and a student of medical problems. In other words, he was research minded.

He was particularly interested in anaphylaxis, and to him goes credit for the initial discovery that an isolated strip of smooth muscle of intestine from a sensitized animal would contract in the presence of antigen. This phenomenon, now known as the Schultz-Dale reaction, was one of the first basic demonstrations of what takes place in the anaphylactic shock reaction of the guinea pig. It clarified the cause of death by asphyxia due to contraction of the smooth muscle of the bronchi.

Thus, it is fitting that the William Schultz lecture should be concerned with the theme of medical research, particularly research in the realm of hypersensitivity.

Of course, we realize that none of us would be practicing the kind of medicine or allergy that we do today if the results of clinical study and research had not been made applicable to certain of our problems. But research is a demanding and fickle mistress. Too often, years of work lead to no tangible results, or so we think, for sometimes a finding or study is passed over because it shows no evidence of usefulness and at the time no one knows quite what to make of it or how to interpret it.

In the fall of 1938 I attended the dedication of the Squibb Institute of Medical Research. Among the various addresses, there was one that made a lasting impression—that by Abraham Flexner whose subject was "The Usefulness of Useless Knowledge." A certain philanthropist, in conversation with

The first William H. Schultz lecture, presented at the 16th Annual Meeting of the American Academy of Allergy, Hollywood-by-the-Sea, Florida, Jan. 11, 1960.

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Dr. Flexner, stated that he intended to leave his vast fortune for the promotion of education in useful subjects, and he cited Marconi as an example of the world's most useful worker in science. "Not at all," was Dr. Flexner's reply. He went on to say that Marconi was merely inevitable; his share in the development of wireless and radio was practically negligible. Credit really belonged to Maxwell, for his abstract equations in magnetism and electricity, and to Hertz, for the demonstration of the electromagnetic waves which carry the signals. Hertz and Maxwell were the geniuses whose apparently useless theoretical work many decades before was seized upon by Marconi and applied by him, to his great fame and fortune. The fundamentally useful men were those who, with no thought of usefulness or of immediate application, were driven by an insatiable curiosity to delve into more basic scientific problems. I am sure that Dr. Flexner was not intending to discredit the application of factual knowledge to useful ends. He was making the point that no one at a given time can know the eventualities, the ultimate effects, of research at some later day. What today seems useless is tomorrow the keystone of a new arch for progress in a novel and unexpected way. All progress is based upon the accumulated knowledge of the past, but occassionally something fundamental is passed over. This happened in the case of Mendel in his work with plants. It seemed petty and superficial that a monk should be toying with forms and colors of flowers. Years passed before his great contribution to genetics attracted the slightest attention. The important point is that basic facts must be correct. Their application may come years later, and often in an unexpected way. Their interpretation may justifiably change with times and circumstances.

All of this is by way of leading up to the topics of research which I want to discuss with you. I am impelled to say, however, that while I earnestly hope that my remarks will prove interesting and perhaps stimulating, nevertheless they do not contain one iota of immediate practical value. I propose to review some of the ideas and some of the research in various fields, particularly those related to the field of allergy, in the hope that they will act as an incentive to greater endeavor in your chosen work.

Curiosity, to my mind, is the most impelling force in investigative thinking. This is especially applicable (in fact it is an essential quality of mind) in one who professes an interest in research. If one studies the types of medical research over the recent past, he cannot help but be impressed by the amount and character of the work devoted to a solution of the mechanisms of disease. The thought is well expressed by Klemperer[1] when he says: "The search for mechanisms to explain the basic alterations of form and function of the body, characteristic for a disease, has become the principle of investigative thinking in Modern Medicine." This involves not only the curiosity mentioned above and other sound qualities of mind, but it entails also the possession of technical skills and sound clinical judgment. As to the skills, one need only mention the many techniques now placed at our disposal—serologic studies as precipitation, complement fixation, hemagglutinations in their many new and refined forms, the separation of serum components by electrophoretic procedures of many types

and the ultracentrifuge, the use of tracer atoms and electron microscopy, and x-ray diffraction and paper chromatography—all resulting from the rapid developments in physical and biologic chemistry, pharmacology, and physics. All of this indicates the need for teamwork. The clinician must be one of the important members of the team investigating a sizable medical problem, for today no one man contains or commands all the necessary skills in these various and varified disciplines.

If we take a bird's-eye view of allergy as it is comprehended today, we are impressed by the fact that, though there has been progress—considerable progress, if you will, both in theory and in practice—this is no cause for smug complacency, for as yet we are still a long way from the heart of our problem. We want to know—we must eventually know—just what it is and why. The idea is well conveyed in the old expression, "What makes Johnny run?" What we do not know is still far greater than the knowledge that we now possess, as far as basic facts and principles are concerned.

Even at this late day, there is no definition of allery that is fully acceptable to practitioners and research workers in the field, and the situation with regard to nomenclature is almost chaotic. Are the words "allergy," "hypersensitivity," and "sensitization" to be used synonymously? Who can be sure that he knows what one writer means when he uses such a term as, let us say, "atopic eczema"? When Coca and I coined the word "atopy," we meant to designate that group of human diseases in which the genetic factor was evident but not known, but we were impressed by the findings in that study on human hypersensitiveness which Vander Veer and I[2] published in 1915. The reason for this confusion is clear and can be tersely summed up in one word—ignorance (a lack of knowledge of the fundamentals of the reaction as it occurs spontaneously or is artificially induced). One thing we can agree on is that, as we see it today, allergy and the allergic state are essentially immunologic problems.

In the search for mechanisms in diseases of allergy or in those diseases in which allergy may play a primary or secondary component role, research along immunologic lines has been in the forefront. It is only one of the special fields, however, for, as just stated, chemistry, physics, bacteriology, pathology, endocrinology, pharmacology, mycology, virology, and others, are involved.

In terms of immunology, we consider the allergic state to be one due to the existence of a specific mediating mechanism that we call antibody. Contact of the proper antigen with its antibody produces the reaction, either somatic or cellular, in the sensitized body. In many instances the existence of the mechanism may be established by the direct skin test or by the transfer of reactivity from a sensitized subject to a nonsensitized normal animal or man. The Prausnitz-Küstner phenomenon of the "immediate" reaction has long been recognized. The demonstrated transfer of the "delayed" or tuberculin type is relatively recent, having been established in animals by Chase in 1947 and extended and amplified by Lawrence in man.

The scope of allergy has been steadily enlarged, however, and the hypothesis of allergy or immunology (or perhaps I should say hypersensitivity), is being used in the hope of unmasking the mechanisms of various diseases of unknown pathogenesis. In line with this, I believe it will not be amiss if I review briefly some of the more recent work on such diseases as acute disseminated lupus, encephalitis, multiple sclerosis, and purpura, bring to your attention the immunologic studies being applied in tissue homotransplantation, and present briefly a new concept of the sensitizing factor in the immediate type of allergy.

First, however, let us pay attention to some work more immediately connected with allergic mechanisms.

Admittedly, we still do not know the exact cause of the abrupt reaction in the immediate allergies. The earliest work was done on the artificially sensitized animal (guinea pig, rabbit, or dog) so, quite naturally, the theories applicable in anaphylaxis were readily applied to the spontaneous sensitizations of man. For that matter, they still are—often with apparent disregard for certain known immunologic differences.

Over the years theories have been proposed to explain the anaphylactic shock response and, at least by inference, applied to the spontaneous allergies, such as hay fever. The original one was the humoral theory, in which the combination of antigen and antibody in the blood or body fluids was thought to result in elaboration of a so-called anaphylatoxin. In many ways this proved inadequate and was replaced by the cellular theory. Here the reaction was assumed to take place between antigen and cellularly fixed antibody. It was a somatic rather than a humoral reaction. This idea, too, has some inadequacies and, while it has been and is quite generally accepted as a fairly satisfying explanation, it is now being challenged by the protease activation theory. Forty years ago Bromfenbrenner,[3] Bordet,[4] and others noted that the interaction of antigen and antibody in a serum caused a sudden reduction in antiproteolytic substance. This released the protease enzyme and caused a rapid proteolysis with resulting autodigestion of serum proteins and liberation of toxic substances which, in turn, produced the symptoms of anaphylaxis.

Recently, Burdon[6] has reviewed the literature and done original work which tends to support an enzymatic basis for anaphylaxis. While he does not claim that activation of protease has been proved and is completely satisfactory, it is an intriguing theory that has to be reckoned with. His modification of Bromfenbrenner's concept of the events in an anaphylatic reaction has been briefly summarized by him: First, there is an antigen-antibody combination involving free or cellular antibodies or both. Next, there is rapid absorption of protease inhibitor with the instantaneous activation of plasma and tissue proteases, the so-called plasmin and its precursor plasminogen. This results in the shock caused by the release of active toxic substances (histamine, heparin, and other products of autodigestion). In other words, the normal protease—antiprotease balance is upset. Apparently, then, this is both a cellular and a humoral reaction, but there are certain basic differences between this and the earlier hypotheses. More will be heard of this, as work is continuing, but we may be sure that as

we delve deeper into these basic reactions enzyme participation will seem to play an increasingly prominent role. However, those of us more directly concerned as clinicians with the spontaneous reactions in man must be wary of accepting or applying the findings in artificially induced sensitization of animals without careful and critical analysis and study.

As I look back from the vantage point of 1960, I see that two important though unrelated events have had a profound effect on the molding of men's minds in the field of medical research. I refer to the broad concept of auto- or isosensitization to explain the pathogenesis of certain diseases of unknown origin. The first was Burkey's successful reproduction of a human eye condition known as endophthalmitis phaco-anaphylactica. The second was the production of an encephalomyelitis by immunologic procedures by Rivers and his associates.[5] In each case, the findings resulted from a study with the limited objective of reproducing a certain type of clinical disease in the hope of discovering something of its mechanism of origin. Each involved the new idea that an animal might, under special circumstances, develop a sensitization to its own or to homologous or heterologous tissues and thus give rise to a focal or general reaction upon proper antigenic contact.

I remember visiting with Burkey in his laboratory at the Wilmer Institute at Johns Hopkins in the late 1920's. He had found it difficult to create a sensitizitation of rabbits to lens protein. It was a very poor antigen. After some six to eight years of trying this and that, he conceived the idea of injecting a lens-protein broth in which he had grown staphylococcus from a strain he called H. A. It was an active producer of an exotoxin. To his delight, animals could be readily sensitized to lens protein after injection with lens-protein broth, and their sera gave high precipitation. This, I believe, was the precursor of the Freund adjuvant so successfully used in more recent years.

Burkey went a step further and found that traumatization of the lens by needling released lens protein in the sensitized rabbits. Shortly thereafter there developed an eye reaction which was clinically and histologically typical of endophthalmitis anaphylactica. In short, antosensitization was demonstrated. The other noteworthy event was the production of an autosensitization reaction in 1933 by Rivers and associates, who had sensitized monkeys with an emulsion of monkey-brain tissue; subsequent injection of the emulsion produced an encephalomyelitic reaction in the monkeys.

Thus was opened up an entirely new approach to the study of disease in animals and, by inference, to diseases of man by the process of auto- or isosensitization.

Now let me review a few of the diseases which may, with reservations, be considered as probable examples in which antosensitization might explain the pathogenesis.

Systemic Lupus Erythematosus.—In no one of the diseases of unknown etiology and pathogenesis have more interesting results been accumulating as a result of research than in disseminated or systemic lupus. It was Klinge[7] who first called attention to the fibrinoid degeneration of collagen and to changes in

ground substance in rheumatic fever and rheumatoid arthritis. He regarded these as evidence of hypersensitivity. As a result, similar structural changes permitted such miscellaneous diseases as scleroderma, dermatomyositis, periarteritis, serum disease, rheumatoid arthritis, and lupus to be classified as the collagen diseases; following Klinge, they were then likewise assumed to be allergic diseases or at least to have an allergic component or an allergic pathogenesis. Klemperer and his associates had long been interested in the hematoxylin staining of tissues found in lupus, and in 1950, after the "L. E." cells were demonstrated, they returned to a study of the hematoxylin-staining tissues which they then found in practically all lupus cases and which were especially abundant in renal and splenic tissues. Hargraves[8] had reported the presence of the hematoxylin cell, the so-called "L. E." cell, in the bone marrow, and shortly thereafter it was found in the circulating blood. These cells were believed to be pathognomonic for lupus. Physical and chemical studies proved that the "L. E." cells were identical with the hematoxylin-staining bodies which Klemperer had described earlier. A further development was the demonstration that the plasma of lupus patients produces the "L. E." cells in vitro from normal bone marrow cells and that this property resides in the electrophoretic gamma globulin. When rabbits were immunized with this globulin, they developed a specific antiglobulin antibody that prevented the lupus serum from producing "L. E." cells in the test tube.

Further studies have shown that a factor in the lupus serum inactivates the inhibitor of the enzyme desoxyribonuclease normally present in leukocytes, whereupon the enzyme then attacks or digests the cellular chromatin which then accepts the stain and becomes a hematoxylin body. This emphasizes what I said earlier concerning the emergence of enzymes as increasingly important factors in disease mechanisms.

In addition, there have been found in lupus cases many abnormal serologic reactions with circulating antibodies in the gamma globulin fraction of the serum. There are agglutamines for red blood cells, for leukocytes, and for platelets, but the antinuclear factors seem most significant since nucleic acid is, of itself, a very poor antigen. That they are auto-antibodies is suggested by the reaction with a patient's own cell proteins.

Here is another study that I have not seen mentioned in the literature. Moolten and Clark,[9] in 1952, reported before the New York Academy of Science that on two occasions they had recovered from a patient with lupus a virus which produced the "L. E." cell phenomenon in vitro. This virus, inactivated by formalin, was injected as a vaccine in a human volunteer who thereupon developed a neutralizing antibody to inhibit the in vitro phenomenon. Does this mean, then, that lupus may be a viral disease? At present it is anyone's guess, but it must be emphasized that I have not found in the literature any confirmation or any refutation of this finding.

Again, in the recent literature there are several reports of lupuslike symptoms produced by drugs. After prolonged Apresoline therapy, three male patients with penicillin sensitivity in one study and thirteen or 139 patients in

another developed signs and symptoms suggesting lupus; "L. E." cells were present in the blood in some cases. I have only touched on a few of the highlights of the studies on the immunologic aspects of lupus, but one thing stands out: Lupus is a "disease of an unusual immunologic responsiveness," as Holman[10] puts it. In spite of the presence of so many different auto-antibodies, however, the part they play in the etiology and symptomatology of lupus remains in question.

Now this still is far from adequate proof of autosensitization as a basic cause in the pathogenesis of lupus unless one proceeds to speculate—and that is not as dangerous as it sounds, for it was speculation that led Burkey and Rivers to experiments that finally demonstrated autoimmunization. Hypothesize as much as you will, as long as you remember that the hypothesis is merely a dream until or unless it is finally demonstrated as fact. Isosensitization is another thing. How is it conceivable that a person can be reactive to his own cells? That "horror autotoxicus" was drummed into us even before anaphylaxis was discovered.

Remembering now that it is speculation, is there not some way in which one can rationalize the concept that isoimmunization might occur? I believe that there is and there are examples in which, as the result of an infectious element, a cell could be so altered as to become a foreign protein while still carrying the function and specificity of the original cell.

I have already spoken of Burkey's use of a bacterial staphylococcic exotoxin to enhance antigenicity of lens protein. That viruses could effect cellular damage and alter antigenicity is illustrated by a relatively recent (1955) report by Netoka and associates, entitled "Relationship of Bacteriophage to Antigenic Changes in Group E Salmonellas." In both of these instances—one involving a bacterial product and the other a viral product—tissues or cells have been so altered that they could conceivably become antigens, thus laying the basis for an auto- or isosensitization.

The Purpuras.—Alexander[11] reported that purpura may be induced in certain patients by food or drugs and he attributed this to a food allergy of the delayed type. Usually these purpuras are of the nonthrombocytopenic type. However, the mechanism has not yet been identified; the evidence rests upon clinical trial and error. It appears now that the conventional classification based upon platelet count no longer holds, for Ackroyd[12] reports severe purpura with normal platelet count and a low count without hemorrhage.

Bedson,[13] in a very neat experiment, reduced the platelets in guinea pigs by injecting an antiplatelet serum. Purpura occurred, for the antiserum not only reduced platelets but also damaged the vascular endothelium. Bedson felt that this latter was the important point, for vascular necrosis and not the reduced platelets per se was the cause of the purpura. Ackroyd has seen the rest of the picture in a patient sensitive to Sedormid. A patch test to the unbroken skin elicited a purpuric lesion. He further showed in this patient's blood a factor which would cause in vitro lysis of platelets from a normal man as well as from the purpuric patient.

Other workers such as Harrington,[14] Hirsch,[15] and Stefanni,[16] have caused typical thrombocytopenic purpura after giving normal human subjects transfusions with blood of purpuric patients. As matters now stand, the picture is not entirely clear but it begins to appear that, as in the case of lupus, the enzyme system may be at fault. This, however, does not exclude sensitization as an etiological factor.

Multiple Sclerosis.—The idea that the disseminated lesions of multiple sclerosis might have a basis in allergy is not entirely new, for they hark back to the work of Rivers and his associates, who produced lesions of acute encephalitis following sensitizing injections of heterologous brain emulsions. In 1947, Morgan, Kabat and associates, and Morrison independently reported producing an acute encephalomyelitis with emulsions of brain cord and nerve tissue, and finally Freund, using a mycobacterium, produced a chronic lesion closely resembling multiple sclerosis.

In his more recent work, Waksman[20] demonstrated the highly selective action of antigen. Rabbits injected with spinal-cord extract responded with lesions of brain cord, and peripheral nerves. Later, using sciatic-nerve antigen, Waksman produced lesions limited to this nerve and its ganglia (a neuronitis), although the cellular changes were those found in the encephalopathies.

Thyroiditis.—Under the leadership of Witebsky and his co-workers,[21] serum studies of human thyroid disease by means of precipitin, tanned-cell hemagglutination, and complement–fixation tests have been yielding results that permit the concept that thyroiditis in its several forms is related to auto-antibody formation, but the amount of antibody is an indication of the extent of autoimmunization and is not the cytotoxic agent itself. Here, as in the other instances cited, there exists a gap between the specific evidences of a disease and its actual cause.

The production of auto-antibody is developing rapidly and, with it, the development of a basis for isosensitization. I have hardly touched the high points, but this is where the studies stand today. Obviously, there is a large gap between these experiments and the clinical disease, for there is yet no proof that a person will spontaneously develop cytotoxic antibodies to his own living tissues. This does not rule out the idea, however, for we have spoken of the synergistic action of bacterial and viral poisons which may play a role in the apparently spontaneous development of auto-antibodies.

Tissue Homotransplantation.—Time permits no more than the briefest mention of this relatively new area. The problem is largely an immunologic one; the rejection of a homologous skin graph has all the aspects of an allergic reaction. Here is an important field to challenge the ingenuity of researchers for years to come.

Skin-Sensitizing Factor.—Now I shall speak very briefly of one area of study that has engaged our attention over the recent years. I refer to the skin-sensitizing factor of spontaneous allergy of the immediate type—in other words, the factor of hay fever.

This mediating mechanism has many unique features that differentiate it from induced antibody: (1) it develops spontaneously under some genetic influence; (2) it is self-perpetuating without antigenic stimulation; (3) it does not bind antigen unless it is bound to a cell; (4) it exists alone without associated antibodies, such as precipitins or muscle-sensitizing antibodies, and often in very high titer; (5) it is not highly specific; (6) it has a fingerprint individuality (in other words, all ragweed-sensitizing antibodies are not exactly alike); (7) it is quite unstable. We find a more important difference, however—induced antibodies are generally considered as part of or carried in some single serum component.

In the several efforts of various experimenters to discover the component in which it belonged, each using a different electrophoretic technique, skin-sensitizing activity was recovered in different serum components all the way from gamma to alpha$_2$. No two agreed. In our work, in which a similar technique was used, we confirmed their findings for the most part. This is not the usual story of separation for induced antibody. This has led us to challenge the conventional acceptance of induced antibody as a stable serum component. Some new concept has to be found to account for the spreading features of this spontaneous skin-sensitizing factor which has so many unconventional attributes. One analogy—highly speculative, to be sure—might picture the skin-sensitizing factor as some coenzyme-like entity, a prosthetic group, which forms loose combinations with a serum protein resembling apo-enzyme which manifests reactivity only when present in proper combinations. Most, if not all, known features of the skin-sensitizing factor can easily be explained on the basis of such a simple enzymatic analogue. This has yet to be proved and is a part of our continuing study.

CONCLUSION

In this sketchy review of immunologic research in the several fields of medicine, I have merely tried to indicate the vast amount of work being done. The opportunities for further effort are still as great as ever—they seem limitedless. Progress is made, but it is slow, and the final answer to just what hypersensitivity and allergy basically are must await the future.

REFERENCES

1. Klemperer, P.: Pathology of Systemic Lupus Erythematosus *in* Progress in Fundamental Medicine, edited by J. F. A. McMannus, Philadelphia, 1952, Lea & Febiger, p. 51.
2. Cooke, R. A., and Vander Veer, A.: Human Sensitization, J. Immunol. **1**: 201, 1916.
3. Bromfenbrenner, J.: The Nature of Anaphylatoxin, J. Exper. Med. **21**: 480, 1915.
4. Bordet, J.: Studies in Immunity (translated by Gay), New York, 1909, John Wiley & Sons, p. 1.
5. Rivers, T. M., Sprunt, D. H., and Berry, G. P.: J. Exper. Med. **58**: 38, 1933.
6. Burdon, K. L.: Project No. 21-3501-004, Report No. 1., U. S. Air Force School of Aviation Med., Randolph Field, Texas, 1953.
7. Klinge, F.: Der Rheumatismus, Berlin, 1933, Springer-Verlag.
8. Hargraves, M. M., Richmond, H., and Morton, R.: Presentation of Two Bone Marrow Elements: The "Tart" Cell and the "L.E." Cell, Proc. Staff Meet., Mayo Clin. **23**: 25, 1948.

9. Moolton, S. E., and Clark, E.: Tr. New York Acad. Sc. **14**: 232, 1952.
10. Holman, H. R., and Kunkel, H. G.: Affinity Between the Lupus Erythematosus Serum Factor and Cell Nuclei and Nucleoprotein, Science **126**: 162-163, 1957.
11. Alexander, H. L.: Allergic Purpura, J. A. M. A. **92**: 2092, 1929.
12. Ackroyd, J. F.: Pathogenesis of Thrombocytopenic Purpura Due to Hypersensitivity to Sedormid (Allyl-isopropyl-acetyl-carbamide), Clin. Sc. **7**: 249, 1949; Mechanism of Reduction of Clot Retraction by Sedormid in Blood of Patients Who Have Recovered From Sedormid Purpura, Clin. Sc. **8**: 235, 1949; Allergic Purpura, Including Purpura Due to Foods, Drugs and Infections, Am. J. Med. **14**: 605, 1953.
13. Bedson, S. P.: Blood Platelets Anti-Serum, Its Specificity and Role in the Experimental Production of Purpura, J. Path. & Bact. **25**: 94, 1922.
14. Harrington, W. J., and others: Immunologic Mechanisms in Idiopathic and Neonatal Thrombocytopenic Purpura, Ann. Int. Med. **38**: 433, 1953.
15. Hirsch, E. O., and Gardner, F. H.: The Transfusion of Human Blood Platelets With a Note on the Transfusion of Granulocytes, J. Lab. & Clin. Med. **39**: 556, 1952.
16. Stefanni, M., and Dameshek, W.: The Hemorrhagic Disorders: A Clinical and Therapeutic Approach, New York, 1955, Grune & Stratton, Inc.
17. Morgan, I. M.: Allergic Encephalomyelitis in Monkey in Response to Injection of Normal Monkey Nervous Tissue, J. Exper. Med. **85**: 131, 1947.
18. Kabat, E. A., Wolf, A., and Bezer, A. E.: The Rapid Production of Acute Disseminated Encephalomyelitis in Rhesus Monkeys by Injection of Heterologous and Homologous Brain Tissue With Adjuvants, J. Exper. Med. **85**: 117, 1947.
19. Morrison, L. R.: Disseminated Encephalomyelitis Experimentally Produced by Use of Homologous Antigen, Arch. Neurol. & Psychiat. **58**: 391-416, 1947.
20. Waksman, B. H., and Adams, R. D.: A Comparative Study of Experimental Allergic Neuritis in the Rabbit, Guinea Pig, and Mouse, J. Neuropath. & Exper. Neurol. **15**: 293-334, 1956.
21. Witebsky, E., and Rose, N. R.: Studies on Organ Specificity. IV. Production of Rabbit Thyroid Antibodies in the Rabbit, J. Immunol. **76**: 408-416, 1956.

REPORT OF THE COMMITTEE ON DRUGS

Data on Tacaryl and Forhistal

DURING 1959 two new antihistamines were evaluated by the group. One of the drugs was provided by Ciba Pharmaceutical Products, Inc., under the code of SU 6518 (Forhistal). A total of 242 patients were tested. Of this group, 202 had hay fever, twenty-eight had perennial rhinitis, eight had asthma and four had urticaria. Among the 202 hay fever patients treated, results were reported as fair in eighty-nine, good in ninety-nine, and excellent in fourteen. Of the perennial rhinitis group, thirteen reported fair benefit and fifteen had good results. Of the asthma group, results were reported as fair in seven patients and good in one. One urticaria patient reported fair relief, and three had good relief. As for side effects twelve patients had minor drowsiness, one complained of a slight degree of nausea, two had dryness of the mouth, three experienced central nervous system stimulation, and one complained of headaches. All side effects appeared to be minimal.

Another drug, supplied by Mead Johnson & Company, is called Methdilazine (Tacaryl) and was supplied in the form of 4 mg. tablets. A total of 498 patients were variously treated. In the group of 420 patients with hay fever, fair results were obtained in 121 patients, good results in 210, and excellent results in eighty-nine. In the perennial rhinitis group, results were fair in twenty-seven cases, good in twenty-one, and excellent in two. Of sixteen asthmatic patients treated for acute exacerbations, twelve reported fair relief and four had good relief. Of the twelve urticaria patients treated, five had a fair response and seven reported good relief. Side effects included slight drowsiness in eighteen patients, moderate drowsiness in two, nausea in two, dryness of the mouth in three, nervousness in one, and headache in three patients. None of these side effects were of a severe nature.

Eight-milligram tablets of Tacaryl were also supplied, and a small group of eighty patients were studied. Among the hay fever patients, results were fair in eleven, good in thirteen and excellent in fourteen. In the group with perennial rhinitis, three patients reported fair relief, six had good relief, and three obtained excellent relief. Three patients with urticaria reported poor relief, and eight obtained good relief. Twelve asthmatic patients reported little or no benefit, and seven patients felt some benefit. Side effects included four cases of moderate

TABLE I. SUMMARY OF DRUG EVALUATION REPORT

DRUG	HAY FEVER			PERENNIAL RHINITIS			ASTHMA			URTICARIA			SIDE EFFECTS									
													DROWSINESS		NAUSEA		DRYNESS OF MOUTH		NERVOUSNESS		HEADACHE	
	F	G	E	F	G	E	F	G	E	F	G	E	SL.	MOD.	SL.	MOD.	SL.	MOD.	SL.	MOD.	SL.	MOD.
Forhistal	202 Cases			28 Cases			8 Cases			4 Cases												
CIBA Su 6518 2 mg. prn q 4 h 242 cases	89	99	14	13	15	0	7	1	0	1	3	0	12	0	1	0	1	0	3	0	1	0
Tacaryl (Methdilazine) Mead Johnson	420 Cases			50 Cases			16 Cases			12 Cases												
4 mg. prn q 4 h 498 cases	121	210	89	27	21	2	12	4	0	5	7	0	18	2	2	0	3	0	1	0	3	0
Tacaryl (Methdilazine) Mead Johnson	38 Cases			12 Cases			19 Cases			11 Cases												
8 mg. prn q 4 h 80 cases	11	13	14	3	6	3	12	7	0	3	8	0	4	4	2	0	1	0	1	0	1	0

F = Fair (O to 60 per cent).
G = Good (60 to 90 per cent).
E = Excellent (90 to 100 per cent).
Sl. = Slight.
Mod. = Moderate.

drowsiness, four cases of slight drowsiness, two cases of mild nausea, one case of dryness of the mouth, one case of central nervous system stimulation, and one case of headache.

It would appear that both Forhistal and Tacaryl (in both the 4 mg. and 8 mg. dosages) are effective antihistamine drugs.

Seymour B. Crepea, M.D.
Chairman, Committee on Drugs
American Academy of Allergy

OBITUARIES

WYNDHAM BOLLING BLANTON

WYNDHAM BOLLING BLANTON, Fellow of the American Academy of Allergy, died in Richmond, Virginia, on Jan. 5, 1960, at the age of 69. He was a past vice-president of the Academy and at different times served on the Executive Committee, the Program Committee, the Nominating Committee, the Committee on Education, the Subcommittee on Undergraduate and Post-Graduate Education, and the Obituary Committee.

Dr. Blanton was born in Richmond in 1890. He received a B.A. degree from Hampden-Sydney College in 1910, an M.A. from the University of Virginia in 1912, and an M.D. from the Columbia College of Physicians and Surgeons in 1916. He did postgraduate work in medicine at Columbia and in Berlin and Edinburgh. He served as a captain in the Army Medical Corps in World War I and was later a senior surgeon in the United States Public Health Service.

He joined the faculty of the Medical College of Virginia in 1930 as an Associate Professor of Medicine. He became Professor of the History of Medicine in 1933. He organized the Allergy Clinic in 1936 and continued as its director until his resignation in 1954. He became Professor of Clinical Medicine in 1939, and Emeritus Professor of Clinical Medicine and of the History of Medicine in 1958.

He was a past president of the Richmond Society of Internal Medicine, the Richmond Academy of Medicine, the Historic Richmond Foundation, the Virginia Historical Society, the Society of Colonial Virginia, and the Virginia Chapter of the Society of the Cincinnati. He was editor emeritus of the *Virginia Medical Monthly,* past associate editor of the *Annals of Medical History,* and contributing editor of the *Journal of the History of Medicine and Allied Science.*

He was a Fellow of the American College of Physicians and of the American Academy of Allergy. He was a member of the American Medical Association and of his county and state medical societies, the American College of Chest Physicians, the American College of Allergists, the American Association of the History of Medicine, the Southern Medical Association, and the American

Clinical and Climatological Association. He was a member also of the Society of Colonial Wars and of Phi Beta Kappa.

He was the author of two textbooks, *Physical Diagnosis* and *Clinical Allergy,* and of four historical works, *Medicine in Virginia in the 17th Century, Medicine in Virginia in the 18th Century, Medicine in Virginia in the 19th Century,* and *The Making of a Downtown Church.*

He was an active churchman, having served as an elder of the Second Presbyterian Church and as chairman of the Board of Trustees of the Union Theological Seminary in Richmond.

Dr. Blanton is survived by his widow, a daughter, and two sons (both physicians, who were associated with him in the practice of medicine).

Oscar Swineford, Jr.

LORAINE ORR DUTTON

Loraine Orr Dutton of El Paso, Texas, died on Jan. 23, 1960. He was a Fellow of the American Academy of Allergy, having been elected in 1939.

Dr. Dutton was born in Waco, Texas, on Aug. 2, 1898. He obtained an A.B. degree at Texas Christian University in 1918, following which he served in the U. S. Army in Siberia. After discharge from the Army he received an M.S. degree from his alma mater and became Director of Laboratories, successively, at the Baptist Memorial Hospital and the Methodist Hospital in Memphis. While serving at the latter hospital, he took a medical course at the University of Tennessee and graduated with an M.D. degree in 1931. From then until the time of his last illness, he was Director of Laboratories and a member of the staff at the El Paso General Hospital and at the Providence Memorial Hospital. He was also Medical Director of the Southwest Blood Bank of El Paso and on the staff of the Hotel Dieu.

Dr. Dutton was a Fellow of the American College of Allergists and of the American Academy of Internal Medicine. He was a member of the Southwest Allergy Forum (president, 1949), the Association of Allergists for Mycological Investigations, the Texas Society of Pathologists, the American and the South Central Associations of Blood Banks, the Southern Medical Association, the American Association for the Advancement of Science, the American Medical Association, and his county and state societies.

Dr. Dutton's chief interests were in the laboratory field of medicine, particularly microbiology. He was also an ardent golfer.

He is survived by his widow and by a son and two daughters.

Howard Osgood

CHARLES NORTON HENSEL

CHARLES NORTON HENSEL of St. Paul, Minnesota, died on April 6, 1959, at the age of 77 years.

Dr. Hensel was an active, public-spirited, and highly respected internist in St. Paul for over fifty years. His special interests were cardiology and allergy.

Dr. Hensel was born in North Dakota in 1882, but he moved to St. Paul as a child. He took premedical and medical courses at the University of Minnesota, obtaining an M.D. degree in 1908. He interned at the Ancker Hospital in St. Paul and took postgraduate work at the Harvard Medical School and in Vienna. He was a staff member at St. Luke's, Miller, St. Joseph's, and Children's Hospitals, his principal activity being at the Miller Hospital. There he conducted the staff meetings with Dr. E. M. Jones for twenty years and was chairman of the medical division of the Hospital Building Fund campaign.

He early became interested in allergy, being a member of the American Association for the Study of Allergy in 1924 and later a Fellow of the American Academy of Allergy. At the time of his death he was Emeritus Fellow of the Academy.

Dr. Hensel was a former president of the Minnesota Society of Internal Medicine, the Minnesota Heart Society, and the Minnesota Academy of Medicine. Besides belonging to his county and state societies, he was a Fellow of the American College of Physicians and the American College of Allergists, a member of the Association for the Study of Internal Secretions, the Minnesota Society for the Study of Diseases of the Heart and Circulation, the Minnesota Pathological Society, the Southern Minnesota Medical Association, the St. Paul Clinical Club, and Nu Sigma Nu.

He was a ready and forceful speaker, and in his earlier years he took part in the University Dramatic Society's presentation of Shakespearean plays.

Dr. Hensel is survived by his widow, a son, a daughter, and eight grandchildren.

The American Academy of Allergy has lost an able and active senior member and extends its condolences to his family.

Howard Osgood

CONNOLLY J. MALLOY

CONNOLLY J. MALLOY, Fellow of the American Academy of Allergy, died in Montreal on Feb. 1, 1960, at the age of 55. His unexpected death, which occurred after a brief illness, came as a shock to his many patients and friends and as a sad loss to his colleagues.

Dr. Malloy studied at McGill University where he obtained a B.A. and later, in 1935, an M.D. degree. He interned at the Royal Victoria Hospital and

at Children's Memorial Hospital in Montreal. He served in the Canadian Army during World War II. At the time of his death he was an Assistant Physician at the Royal Victoria Hospital and Lecturer in Medicine at McGill University.

He was a Fellow of the Royal College of Physicians, the International Association of Allergology, the American College of Physicians, and the American College of Allergists and a member of the Canadian Medical Association, New York Sciences, and the American Association for the Advancement of Science. He took an active interest in the Canadian Academy of Allergy and was instrumental in the founding of the Association of Allergists of the Province of Quebec, of which he was vice-president.

He had a scholarly knowledge of the classics, which was reflected in his publications. Always genial, he had a ready smile and a pleasant manner, and he carried the mark of a gentleman.

Dr. Malloy is survived by his widow, Helga, and by his three children, Brenda, Kirsten, and Brian, to whom the Academy extends its deepest sympathy.

Bram Rose

LESTER CLAIRE TODD

Lester Claire Todd, of Charlotte, North Carolina, died on Dec. 29, 1959. Dr. Todd became a member of the American Academy of Allergy in 1941, retiring to inactive status in 1959.

Dr. Todd was born in Center Point, Iowa, on Sept. 6, 1888. He received an A.B. degree from Tabor College, following which he served for two years as supervisor of twenty-five government schools in the Philippine Islands. Returning to the United States, he obtained an M.D. degree in 1918 at the University of Michigan Medical School. His lifelong interest in laboratory work was forecast by his work in research for Parke, Davis & Company during his summer vacations. He saw active service in the U. S. Navy Medical Corps for a number of years and held the rank of captain when he left the service.

Dr. Todd was director of laboratories and of pathology at the Crowell Clinic and pathologist for the Charlotte Sanatorium. He was Consultant in Allergy at the Charlotte Memorial Hospital and Consultant in Dermatology at the Presbyterian Hospital. He had practiced allergy since 1930.

He was a Fellow of the American College of Allergists and a member of the Southeast Allergy Association, the Southern Medical Association, the American Society of Clinical Pathologists, the Tri-State Medical Association, Phi Chi, and Alpha Omega Alpha, as well as of his county and state societies. He was an elder of the Covenant Presbyterian Church and an honorary life member of the Charlotte Lions Club.

Dr. Todd published many papers in the field of medicine and allergy, and his meticulous attention to detail in his work was noteworthy.

Dr. Todd is survived by his widow, a son, and two grandchildren.

Howard Osgood

A REQUEST FROM THE OBITUARY COMMITTEE

In the past it has been very difficult to learn of the deaths of members of the American Academy of Allergy promptly enough to publish timely obituaries or to communicate with the families of these members.

Any of our readers who learn of the death of an Academy member are earnestly requested to notify the undersigned or the Executive Office of the Academy immediately.

Howard Osgood, Chairman
Obituary Committee
American Academy of Allergy.

ANNOUNCEMENTS

Mid-South Allergy Forum

At a recent meeting, the following officers for the current year were elected by the Mid-South Allergy Forum at Memphis, Tennessee.

President, Lloyd Crawford, M.D.
Secretary-Treasurer, Oliver S. Matthews, M.D.

Allergy Residency Available

Two approved residencies in allergy are available in July, 1960, at Long Beach Veterans Administration Hospital. This is a Deans' Committee Hospital. The program is directed by Dr. Kent Thayer; consultants are Dr. Geo. Piness and Dr. Ralph Bookman. Requirements and stipends are those for all Veterans Administration residencies.

Please address inquiries to Director of Professional Services, Veterans Administration Hospital, 5901 E. Seventh St., Long Beach 4, Calif.

Fellowships Available at University of Virginia Medical School

Fellowships are available in the Allergy-Arthritis-Chronic Lung Disease Division of the Department of Internal Medicine at the University of Virginia Medical School, Charlottesville, Virginia, starting July 1, 1960. There is more emphasis on allergy than on arthritis or chronic lung disease. The stipend is $2,400, plus maintenance in the interns' quarters. The Fellowship is approved by the Board of Internal Medicine and by the Allergy Certifying Board. Candidates should have had two years of training in internal medicine.

Inquiries should be directed to Oscar Swineford, Jr., University of Virginia Hospital, Charlottesville, Virginia.

Fellowship in Pediatric Allergy at NYU–Bellevue Medical Center

A fellowship in pediatric allergy will be available July 1, 1960, in the Department of Pediatrics, New York University–Bellevue Medical Center, College of Medicine. The program includes instruction in procedures and research methods utilized in the field of pediatric allergy. The Fellow will participate in current research activities and attend clinical sessions at Bellevue and University Hospitals. The training is designed to prepare candidates interested in research and in pediatric allergy as a subspecialty. Applicants must have a minimum training of 2 years in pediatrics. Salary will be $4,500 to $5,000.

Direct correspondence to Vincent J. Fontana, M.D., NYU–Bellevue Medical Center, 550 First Avenue, New York, N. Y.

International Symposium on Allergy

An international symposium on allergy, organized by the European Academy of Allergy in collaboration with the Spanish Society of Allergy, will be held in Barcelona, Spain, on June 3-4, 1960.

The subjects discussed will be (1) an evaluation of the diagnostic methods used in allergy, and (2) functional exploration of the respiratory and cardiovascular systems in asthma, emphysema, and allied conditions.

All matters concerning travel and hotel reservations should be arranged with Cook and Co., Pa. de Gracia 1-10, Barcelona 7, Spain, or at the Cook agencies in the different countries (Cook and Co. has been named official agent).

For further information, write to the secretary: Mr. Camps, Academia de Ciencias Medicas, Via Layetana 31, Barcelona 3, Spain.

The 6th International Congress of Internal Medicine

The Congress will meet on August 24-27, 1960, in Basle, Switzerland. It has been organized by the International Society of Internal Medicine (President: Sir Russell Brain, F.A.C.P. [Hon.], London). The president of the Congress will be Prof. Dr. A. Gigon, Basle, and the secretary, Prof. Dr. H. Ludwig, Basle.

The principal subjects of the Congress will be "Pathogenesis and Therapy in Edema" and "Enzymic Regulations in the Clinic." Panel discussions will be on interstitial nephritis, nephrosis, diuretics, diagnostic value of enzymes, therapeutic indications of enzymes, serology in rheumatic diseases, and instruction in internal medicine. There will also be lectures on unannounced subjects. Seventy leading internists from all over the world will take part in the main scientific program by reading papers or joining in the panel discussions. The official languages will be German, French, and English (there will be simultaneous translations for the principal lectures).

Programs and registration forms for the Congress may be obtained from the secretary of the 6th International Congress of Internal Medicine, Dr. H. Ludwig, Steinentorstrasse 13, Basle 10, Switzerland. Registrations should reach the secretary, if possible, by April 30, 1960.